HABITUATION AND DESENSITIZATION AS METHODS FOR REDUCING FEARFUL BEHAVIOR IN SINGLY-HOUSED RHESUS MACAQUES

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SUMMARY

Operant conditioning using positive reinforcement techniques has been used extensively in the management of nonhuman primates in both zoological and laboratory settings. Based on a large body of previous research that demonstrates the utility of such techniques in reducing stress, abnormal behavior, and aggression, this research project was intended to develop and test the usefulness of habituation and counter-conditioning techniques in reducing the fear-responses of singly-housed male rhesus macaques living in the laboratory environment. Additionally, we investigated the variable of temperament as it relates to the reduction of fear-responsivity and overall training success. Based on a Wilcoxon Matched-Pairs Sign Test, we found that animals exposed to desensitization training were significantly likely to show a reduction in the rate at which they engaged in cringing toward humans (exact significance = .016, one-tailed, N – ties = 6), cringing in general (exact significance = .016, one-tailed, N – ties = 6), and in stress-related behaviors (exact significance = .016, one-tailed, N – ties = 6). Animals exposed to basic husbandry training or exposed to no training at all were not significantly likely to show a reduction in the rates of these behaviors. When these same behaviors were analyzed in terms of duration of behavior, desensitization-exposed animals were significantly likely to show reduction in the amount of time spent cringing toward humans (exact significance = .016, one-tailed, N – ties = 6), but not in cringing behaviors in general or in stress-related behaviors. Neither the husbandry-exposed group nor the group exposed to no training showed a significant number of subjects exhibiting a reduction in duration of any of these behaviors. Additionally, initial temperament assessments were found to significantly predict the relative ability of subjects exposed to training to acquire trained behaviors such that animals generally ranked as more inhibited in terms of temperament also ranked as "slower" learners based on a Wilcoxon Matched-Pairs Signed-Ranks test, $z = -.316$, $p = .752$ (two-tailed). Results of this study could enhance both laboratory animal welfare and laboratory animal research, and could be a first step in developing techniques for reducing fearful behavior in rhesus monkeys in the laboratory environment.

CHAPTER 1

INTRODUCTION

Fear has been defined, generally, as “a feeling of agitation and anxiety caused by the presence or imminence of danger” or “a state or condition marked by this feeling” (American Heritage Dictionary, 2000). Fear can also be defined as a state of motivation that normally gives rise to defensive behavior or escape (McFarland, D., 2006). Of course, for animals in captivity, such defensive behavior or escape is often not possible. The animal may struggle to cope with fearful stimuli, and may fail either due to the intensity of the stimulus, or because the animal is prevented from making an appropriate response, such as flight (Hediger, H., 1950). In 1994, John Webster defined the welfare of an animal as its “state as it seeks to cope with its environment.” In his view, an animal attempts to cope either by adapting physiologically (e.g. shivering when confronted with cold temperatures) or behaviorally (e.g., curling up or huddling). When the animal cannot respond appropriately, effects can include severe impairment of the physiological stress response, profound immunosuppression, and behavioral abnormalities (Webster, J., 1994). This is analogous to a state of learned helplessness, first described by Seligman in 1967 (Overmier, J.B. & Seligman, M.E.P., 1967; Seligman, M.E.P., & Maier, S.F., 1967). When animals are confronted with uncontrollable trauma, ‘learned helplessness,’ a behavioral state associated with weight loss, anorexia, norepinephrine depletion, increased passivity, reduced learning, and increased overall stress, often results (Weiss, J.M., 1968; Seligman, M.E.P., 1972; Weiss, J.M., Stone, E.A., Harrell, N., 1970).

To reduce such effects on captive animals, we often cannot always readily change the structural aspects of the animals' environments. For example, in a laboratory environment, it simply may not be possible to alter the animal's caging, or to eliminate the presence of loud and unpredictable noise, or to decrease the frequency of medical procedures. However, we may be able to alter the animal's reaction to fear-provoking stimuli. By using the principles of operant and classical conditioning, we may be able to desensitize or habituate fearful animals to some of the fear-provoking stimuli that they encounter on a daily basis. In this way, we may be able to reduce some of the harmful effects of the fear response that animals in research laboratories often exhibit.

Desensitization is generally described as a process by which the active pairing of a positive reinforcer with a negative event causes the negative event to slowly lose its ability to adversely influence behavior (Chance, P., 2003). This active process is also called counter-conditioning. Systematic desensitization has been used successfully to treat human phobias, such as fear of flying, fear of public speaking, and fear of spiders, and has also been used to reduce the intensity of Post-Traumatic Stress Disorder in veterans of the Vietnam War (Chance, P., 2003). Behavioral therapy for human anxiety, when it incorporates desensitization, often utilizes trained relaxation procedures to compete with the conditioned fearful response. For animals, a similarly competing response could be eating. Using competing responses to interfere with a fearful response is an integral part of the systematic desensitization process as defined by Wolpe (1958, as cited by Callen, E.J., & Boyd, T.L., 1990).

Habituation is a passive process, in which simple exposure to a specific stimulus does, over a period of time, result in decreased response to that stimulus (Webster, J., 1994).

Other methods which have been used with variable success to reduce fear response include pharmacological treatment (e.g., Davis, M., 1986), extinction (with and without pharmacological enhancement), in which a fear-inducing conditioned stimulus is repeatedly presented without the unconditioned fear-inducing stimulus (Walker, D.L., Ressler, K.J., Lu, K., & Davis, M., 2002), and flooding, or response-prevention, which is a form of extinction in which the subject is prevented from making an avoidance-response when presented with a fear-inducing stimulus (Baum, M., 1969; Mineka, S., & Kier, R., 1983; Baum, M., 1988). An unconditioned stimulus, in this case, is one that elicits a response without prior learning, while a conditioned stimulus elicits a response only after being paired with an unconditioned stimulus that elicits that response (Martin, G., & Pear, J., 2002).

In some cases, a combination of habituation and desensitization has been used. For example, Goldstein (1969) found that using habituation (he termed this "progressive approach") and counter-conditioning (or desensitization) together reduced the fear response of 10 Cebus monkeys more than either procedure by itself (Goldstein, A.J., 1969). By using a combination of habituation and systematic desensitization techniques, we may be able to reduce fear responses in laboratory housed rhesus macaques, which would benefit both the animals and the researchers working with those animals.

At the Yerkes Primate Center, monkeys coming into single-housing conditions from larger corral-style housing often exhibit a strong fear response in their new situation. For example, these animals may startle easily, freeze, or press themselves into far corners of the cage when people enter the area. Additionally, some animals that have been in single-housing for longer periods of time exhibit similarly exaggerated fear responses.

The anecdotal observation of the fear-responsivity in these animals is supported by studies that have shown significant, long-term changes in physiological and behavioral measures of stress and anxiety in rhesus macaques moved into single-housing caging in laboratories (Lilly, A.A., Mehlman, P.T., & Higley, J.D., 1999).

It is possible that the variation in fear behaviors observed in singly-housed rhesus macaques is due to temperament differences between fearful and less fearful animals (Suomi, S.J., & Novak, M.A., 1991; Jones, R.B., 1997). Temperament and or "personality" differences have been associated with age, sex, rank, physiological and neurological differences (Boissy, A., 1995; Clarke, A.S., & Boinski, S., 1995; Kalin, N.H., Larson, C., Shelton, S.E., & Davidson, R.J., 1998; Kalin, N.H., Shelton, S.E., Davidson, R.J., & Kelley, A.E., 2001), and temperament ratings have been found to be reliable and consistent (Suomi, S.J., & Novak, M.A., 1991), both across the life-span (Kalin, N.H., & Shelton, S.E., 1998) and across some widely divergent species, such as the stumptail macaque and the zebra finch (Figueredo, A.J., Cox, R.L., & Rhine, R.J., 1995).

Temperament tests have been conducted in rhesus macaques, and animals which were identified as "inhibited" were less readily trained to cooperate with operant conditioning procedures than were animals identified as "moderate" or "exploratory" (Coleman, K., Tully, L.A., & McMillan, J.L., 2005). Rhesus macaque temperaments have also been found to interact with the effectiveness of environmental enrichment programs in improving problem-solving behaviors (Schneider, M.L., Moore, C.F., Suomi, S.J., & Champoux, M., 1991). Researchers have found general differences in "reactivity" levels between different strains of rhesus macaques (Champoux, M., Higley, J.D., & Suomi, S.J., 1996) and between different species of nonhuman primates, such as the baboon and macaque (Heath-Lange, S., Ha, J.C., & Sackett, G.P., 1999). Variation in fear response has also been attributed to rearing differences, such that generally, animals who experienced decreased quality of maternal care have shown increased response to fear stimuli, both physiologically (Parr, L.A., Winslow, J.T., & Davis, M., 2002; Sanchez, M.M., Noble, P.M., Lyon, C.K., Plotsky, P.M., Davis, M., Nemeroff, C.B., & Winslow, J.T., 2005) and behaviorally (Fahlke, C., Lorenz, J.G., Long, J., Champoux, M., Suomi, S.J., & Higley, J.D., 2000; Rosenblum, L.A., Forger, C., Noland, S., Trost, C., & Copland, J.D., 2001;. Parent, C., Zhang, T., Caldji, C., Bagot, R., Champagne, F.A., Pruessner, J., & Meaney, M.J., 2005).

Certain aspects of the laboratory environment have been suggested as being particularly fear-provoking stimuli for rhesus macaques. For example, even non-invasive aspects of the routine husbandry procedures in laboratory facilities (such as cleaning, moving caging, personnel entering animal rooms, etc.) have been found to activate the stress response in rats, mice, rhesus macaques, hamsters, rabbits, bats, geese, starlings, hens, ducks, and sparrows (Balcombe, J.P, Barnard, N.D., & Sandusky, C., 2004). Additionally, the relationship between caretaker and animal has been found to correlate with stress levels in laboratory housed stump-tail macaques (*Macaca arctoides*) (Waitt, C., Buchanan-Smith, H.M., & Morris, K., 2002), and aggression/wounding rates have been found to positively correlate with levels of human activity in laboratory housed chimpanzees (Lambeth, S.P., Bloomsmith, M.A., & Alford, P.L., 1997). It has also been found that rhesus macaques in laboratories show an elevated stress response to being boxed in transfer cages, an effect that is still observed when the animals are being subjected to venipuncture and acute restraint both in the home cage and in the transfer cage (Clarke, A.S., 1987; Line, S.W., Clarke, A.S., & Markowitz, H., 1987).

Also, there is some controversy regarding the placement of laboratory animals, such as the rhesus macaque, in low caging such as is used in the double tier caging system in most laboratory facilities (Schiff, W., Caviness, J.A., & Gibson, J.J., 1962; Buchanan-Smith, H.M., Shand, C., & Morris, K., 2002; Reinhardt, V., & Reinhardt, A., 2000). Placement of animals on the lower tier of two-tier caging racks could affect the fearfulness of some animals. For instance, rhesus macaques have been found to demonstrate persistent fear responses to the optical stimulus of looming, such as could be experienced particularly in the lower racks of two-tiered caging (Schiff, W., et al., 1962). However, two studies have provided fairly convincing data that have not supported the claim that lower-tier housed animals show significant behavioral differences from upper-tier housed animals (Schapiro, S.J., Stavisky, R., & Hook, M., 2000; Schapiro, S.J. & Bloomsmith, M., 2001).

Desensitization and habituation procedures have been used successfully in a number of different settings to enhance animal care. Operant conditioning, an active process in which animals are reinforced for their behavior, and which includes desensitization, is used in many zoos and laboratories to reduce the stress of various management procedures (Laule, G.E., Bloomsmith, M.A., Schapiro, S.J., 2003; Prescott, M.J., & Buchanan-Smith, H.M., 2003), to reduce abnormal behaviors (Laule, G.E., 1993), to enhance animal-caretaker relationships (Savastano, G., Hanson, A., & McCann, C., 2003), to reduce aggression surrounding feeding (Bloomsmith, M.A., Laule, G.E., Alford, P.L., & Thurston, R.H., 1994; Schapiro, S.J., Bloomsmith, M.A., & Laule, G.E., 2003), and to increase affiliative behaviors between conspecifics (Schapiro, S.J., et al., 2003). The benefits of using positive reinforcement training techniques have been posited to include amelioration of some of the aversive physiological and behavioral effects of invasive procedures such as blood draws and venipuncture in nonhuman primates (Elvidge, H., Challis, J.R.G., Robinson, J.S., Roper, C., & Thorburn, G.D., 1976; Reinhardt, V., Cowley, D., Scheffler, J., Vertein, R., & Wegner, F., 1990; Reinhardt, V., Liss, C., & Stevens, C., 1995) and shearing in domesticated sheep (for a review, see Hargreaves, A.L., & Hutson, G.D., 1997). For example, it has been shown that marmosets show reduced stress response to mildly stressful, routine husbandry procedures following positive reinforcement training (Basset, L., Buchanan-Smith, H.M., McKinley, J., & Smith, T.E., 2003; McKinley, J., Buchanan-Smith, H.M., Bassett, L., & Morris, K., 2003). Callitrichids and cebids (both New World monkey species) have been demonstrated to show reduced aggression towards care-staff following operant conditioning training for basic husbandry procedures (Savastano, G., et al., 2003). This is an active field of study, with many continuing lines of investigation, hoping to elucidate the various ways in which operant conditioning can continue to enhance captive management strategies for nonhuman primates.

In this study, we hoped to determine the relative effects of desensitization, basic behavioral training (or husbandry-related training), and habituation (as it naturally occurs over the period of time the animal spends in the environment) on fearful behavior in singly-housed rhesus macaques. We hypothesized that desensitization techniques would be more effective in reducing fearful behavior than basic behavioral training or simple habituation. We also hypothesized that the initial temperament ratings of the subjects would predict both training success and overall effectiveness of treatment.

CHAPTER 2

METHODOLOGY

Subjects

To develop a desensitization-habituation program for a group of macaques, we first identified a group of animals as “fearful.” Subjects were identified by caretakers and animal management staff based on their observed behavior after at least four months at the Yerkes main center facility. Fearful behavior included behaviors typically used to classify fearful behavior in animals, such as freezing, scanning, vigilance, and increased startle (Davis, M., 1992). Only male macaques in single-housing that were 3 years old or older at the start of the project were considered for the study. Once a list of possible subjects was completed, the animals’ current research protocol assignments were considered and discussed with the primary investigators for these protocols and with the veterinary staff at the center. All subjects were currently part of research protocols that were of the same class regarding invasive techniques and procedures. Ultimately, a total of 18 subjects, all male, between the ages of 3 and 7, were identified and selected for the study (see Table 1). All but three of these animals were mother-reared.

Table 1: Subject Table

Animal ID	Gender	Birth	Arrival at MC	Rearing	Treatment group	Room location	Cage location: top/bottom	Protocol class
RUw8	male	Mar-02	Jan-06	Mother	2	Q5	top	D
ROw8	male	Mar-02	Jan-06	Mother	3	Q5	top	D
RBb9	male	Apr-02	Jan-06	Mother	1	Q5	top	D
RLl9	male	Jun-02	Jan-06	Mother	1	Q5	bottom	D
REn9	male	Feb-03	Jun-05	Mother	3	Q6/V153	top/bottom	D
RAc10	male	Jun-03	Mar-06	Mother	2	D115	bottom	D
RBy9	male	May-03	Mar-06	Mother	1	D115	top	D
RFp9	male	Apr-03	Mar-06	Mother	3	D115	top	D
FNv9	male	May-03	Mar-06	Mother	1	D115	bottom	D
RIu10	male	Jun-04	Jun-04	Nursery	3	V158	top	D
RJs10	male	May-04	May-04	Nursery	2	V158	top	D
RSg9	male	May-02	Aug-05	Mother	2	242	top	D
RPn7	male	Apr-00	Nov-01	Mother	2	D126	top	D
RZs8	male	Jul-01	Sep-05	Mother	1	V154	bottom	D
RFt8	male	Aug-01	Sep-05	Mother	3	V153	top	D
RNe9	male	Apr-02	May-02	Nursery	2	RB4	bottom	D
RMc9	male	Apr-02	Jun-05	Mother	1	V159	bottom	D
RAo9	male	Mar-03	May-05	Mother	3	V159	bottom	D

Methods

Once selected for the study, animals were randomly assigned to 3 groups of 6 animals each. These three groups were defined as follows: 1) animals to receive desensitization/habituation training (Group 1); 2) animals to receive general behavioral husbandry training for matched duration sessions (such as target, sit at front of cage, etc.) (Group 2); 3) a control group, in which no training was provided to the animal, but in which habituation was assumed to take place (Group 3).

Because we know of the possible correlations between temperament and the fear response and between temperament and trainability, all subjects were given a basic temperament assessment (Coleman, K., et al., 2005). This test involved each subject receiving a score under the conditions of indirect eye contact, direct eye contact, introduction of a novel food, and introduction of a novel item (see Appendix A). The person conducting the test rates the animal's response qualitatively and adds a latency variable for recording the amount of time it takes for the animal to inspect, touch, manipulate, and in the case of the food, begin to eat, the object or food item. Temperament assessment scores were tabulated for each animal based on the assignation of points in a 5-point Likert scale arrangement where 1 equals least fearful and 5 equals most fearful. Each subject received a temperament test before baseline behavioral data collection began, and again after the treatment phase of the study. Test scores were compared for reliability purposes, and the initial temperament rating of each animal was used for predictive purposes. Initial temperament ratings were based on the same scoring system as had been used previously with this test format by Coleman et al. (2005). The primary investigator conducted all temperament tests.

Additionally, a Response Test was conducted for each subject to measure that animal's individual response to specific stimuli. These stimuli were selected based on discussion with animal care staff regarding possible fear triggers that animals were exposed to daily and which would be consistent across all animals. These stimuli consisted of the following: 1) insertion of the chow stick, which is a 27" long metal pole with a flat scraper device on one end, into the cage to check the Lixit (a water-dispensation device at the back of the cage); 2) offer of a food item to be hand fed at the front of the cage; 3) spraying of the floor of the room with a hose; and 4) removal of excess chow from the cage using a chow stick. Responses were coded across a range of extremely fearful to not at all fearful based on a 7-point Likert scale system where 1 equals least fearful and 7 equals most fearful (see Appendix B). Two raters scored tests with an Inter-rater Reliability score of 92.8 percent. Response tests were conducted before baseline data collection began, and were then repeated at the conclusion of the treatment phase. The first tests were conducted by the primary investigator, who was at that time new to the animals, and the second tests were conducted by a different staff member that the animals were not familiar with and who was trained to conduct the tests in the same exact way. This was done to control for the possible effect of habituation to the primary investigator on Response Test scores. All tests were videotaped and scoring was conducted using the videotaped tests.

During Phase 1 of the study (baseline or pre-treatment), twelve sessions of half-hour observations were conducted per subject to record baseline behavior. These observations were balanced for time of day as much as possible, consisting essentially of 3 hours of observation during "quiet" time and 3 hours of observation during basic husbandry routines. Behavior was recorded in a continuous fashion based on a modified version of an exhaustive ethogram developed by Kate Baker (see Appendix C). Prioritized behaviors were recorded for each animal on a continuous basis to allow for the analysis of both frequency and duration of all behaviors. Additionally, the location of the animal was recorded continuously as back half or front half of the cage. Possible fear triggers (loud noise, approach of human, addition of food or enrichment to cage, spraying of hose, and insertion of the chow stick) were also recorded whenever they occurred during observation sessions. Behaviors indicative of anxiety such as yawning, scratching, and body shaking, were analyzed as stress-related behaviors (Maestripieri, D., Schino, G., Aureli, F., & Troisi, A., 1992; McCormack, K., Sanchez, M.M., Bardi, M., & Maestripieri, D., 2006). Cringing (pressing the body to the floor or wall of the cage in a rigid manner), freezing, fear-grimacing, and screaming were the primary fearful behaviors recorded by the ethogram (see Appendix C for details). All behavioral data was collected by videotaping the animals and coding behavior from the videotapes.

During the Treatment Phase of the study, animals in Group 2 received basic husbandry training (e.g., target training, presentation of body parts) and animals in Group 1 received the same amount of time of fear-reduction training based on the principles of systematic desensitization and habituation. For animals in Group 2, training started with clicker –training (teaching the animal to associate the sound of a metal click with delivery of a food treat), progressed to target training (teaching the animal to touch a short length of plastic tubing when it is placed near the front of the cage), and the remaining behaviors were trained concurrently. For animals in Group 1, clicker-training also came first, and following that, the stimuli tested in the Response Test were organized on an individual basis from least fear-provoking to most fear-provoking, and desensitization to these stimuli then made up the body of the training. For each animal, training began with the least fear-provoking stimuli, leading up to the most fear-provoking as the final training step. A desirable food treat would, for instance, be paired with insertion of a chow stick into the cage in stages, starting with keeping the stick to the outside of the cage, then slowly moving it into the cage, and ending with using the stick in the manner that most caretaker staff employ it. The speed of the training process was in each case determined by the animal's reaction. For example, an animal that was still showing an excessive fear response to one stage of the process would not be advanced to the next stage until a fearful response was no longer observed to that stimulus at that stage (e.g., animal ceases to fear grimace when chow stick is placed one inch from the mesh caging). For both

Groups 1 and 2, training sessions lasted 5 minutes each for a period of six weeks, totaling 25 training sessions per animal. The primary investigator conducted all training sessions. Group 3 received no treatment (see Appendix D for training procedure details).

Following the treatment phase of the study, another period of data collection (Phase 2, or post-treatment) began, organized exactly as the first baseline period of data collection. Additionally, immediately following the conclusion of treatment, the second temperament tests and Response Tests were conducted for each subject.

CHAPTER 3

RESULTS

Behavioral Data Analysis

Behavioral data was analyzed based on the rate of behavior (instances of the behavior per minute) and on the duration of the behavior (percent of each half-hour interval dedicated to the behavior). Within each of these categories, behaviors were first considered as total instances of the behavior, regardless of social modifiers. In a second stage of analyses, fearful behaviors as directed toward human interactants were considered. This allowed for some assessment of the animals' fearfulness behavior in general and, separately, fearfulness as directed toward humans. For all methods of analyses, if a behavior was not recorded to have occurred in more than one subject in any one phase of the study, it was eliminated. This did not result in elimination of any of the fearful or stress-related behaviors, which were of primary interest in this study.

Rate of Behavior Analyses

Rates of behavior were collapsed into the following categories: 1) Aggression (bobbing, cage shaking, open mouth stares, ear flicking, lunging, grabbing); 2) Cringing (cringing, freezing); 3) Abnormal (self-slap, self injurious behavior, head toss, urine drink, feces paint, masturbate); 4) Affiliative (coo, affiliative contact, attempt to touch, lip smack); 5) Submissive (present) 6) Stress (yawning, body shake, scratch). The categories relevant to fearfulness (Cringing and Stress) were then analyzed.

Our sample size was small (n = 6 in each group), and we did not have, in all cases, data that met the assumptions of normality required for parametric analyses. Based on sample size and the distribution of our data, the Wilcoxon Matched-Pairs Sign Test was used to assess the changes in behavior for each treatment group independently (Siegel, S., 1957; Blair, R.C., & Higgins, J.J., 1985). Exact test statistics are reported as they are more appropriate for smaller sample sizes (Bergmann, R., Ludbrook, J., & Spooren, W.P.J.M., 2000).

The results of a Wilcoxon Matched-Pairs Sign test for changes in cringing behavior as directed toward humans revealed a significant number of animals exhibiting a decrease in this behavior in the desensitization group (exact significance = .016, one-tailed, N – ties = 6) (Figure 3). This result was identical for the desensitization group in regards to cringing in general (exact significance = .016, one-tailed, N – ties = 6) (Figure 1), and stress-related behaviors (exact significance = .016, one-tailed, N – ties = 6) (Figure 2, Table 3). Neither of the other two treatment groups (husbandry or habituation/control) showed a significant number of animals decreasing in any of the analyzed behaviors (Figure 1 – 3, Table 4 - 5). Medians and percentiles are reported in Table 2.

Figure 1: Rate of Cringing Behavior across Phase

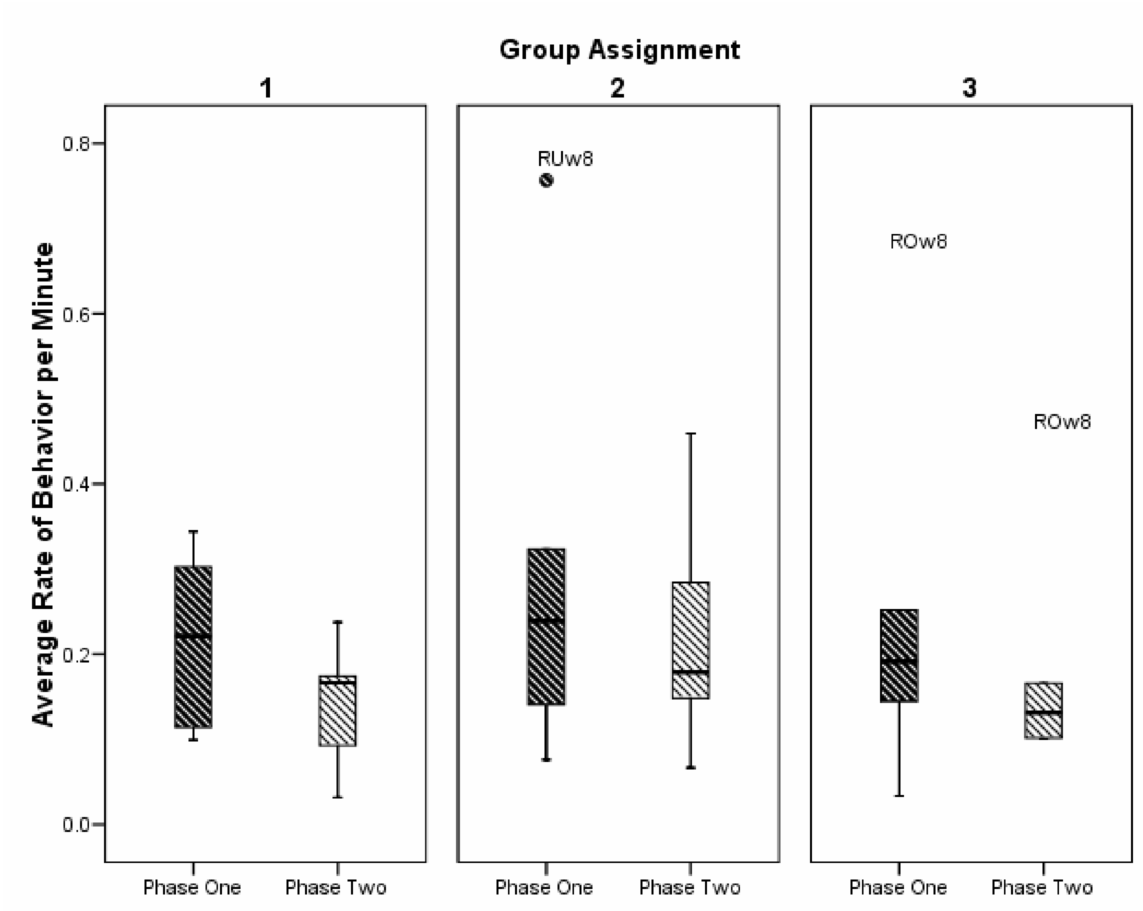


Figure Two: Rate of Stress-Related Behavior across Phase

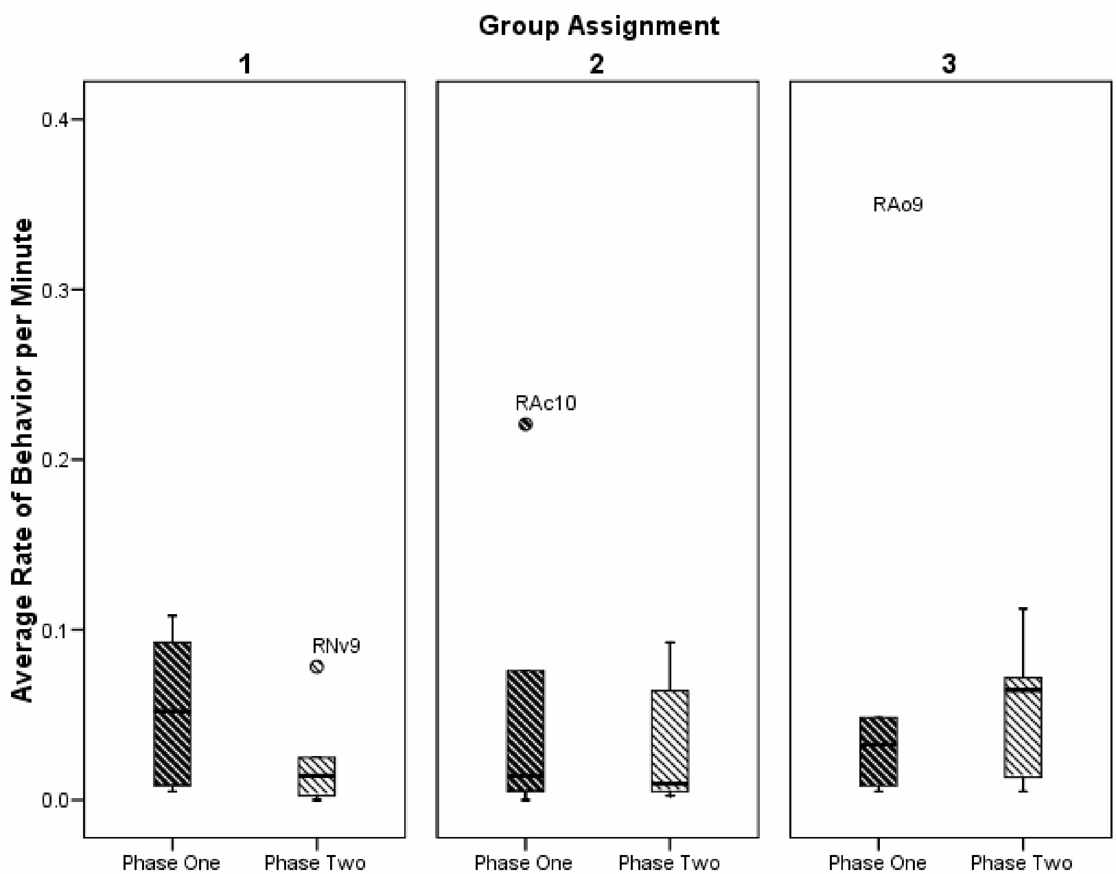


Figure Three: Rate of Cringing Behavior Directed toward Humans

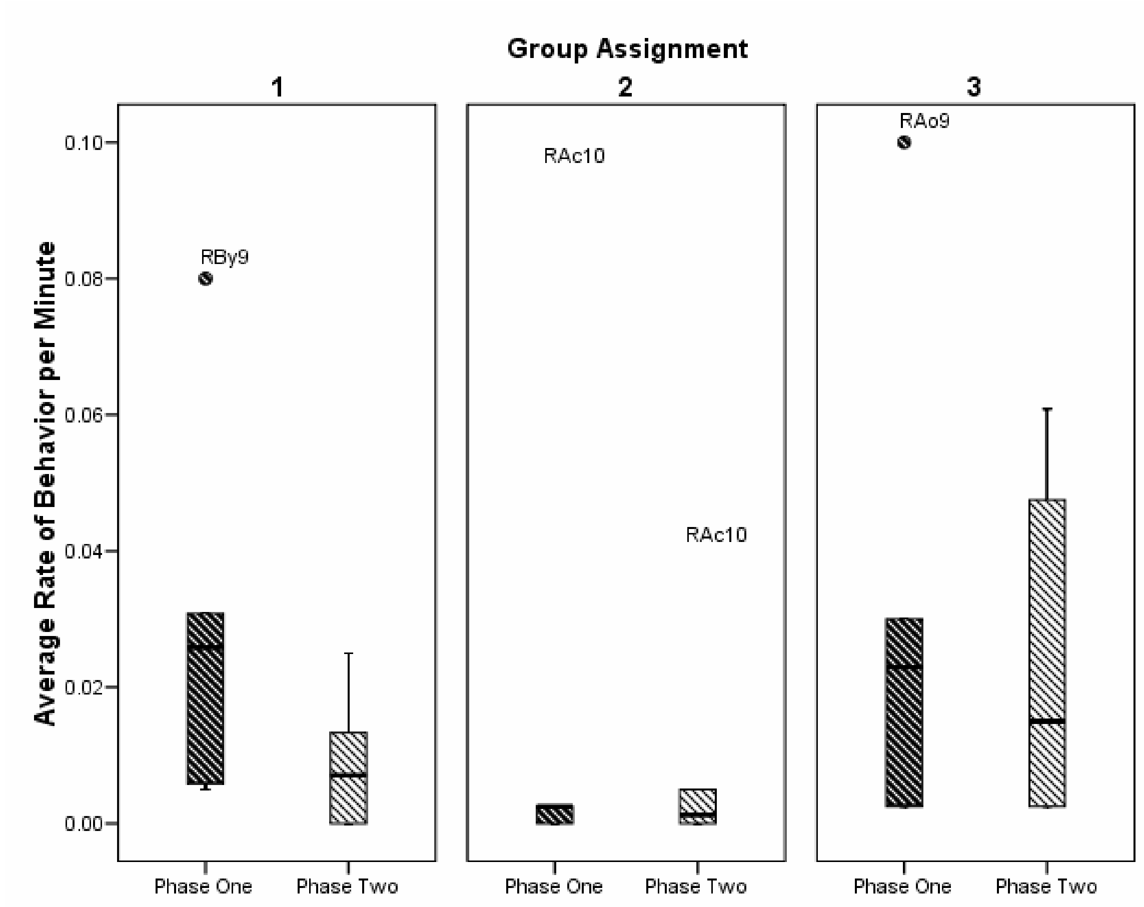


Table Two: Medians and Percentiles for Rates of Behavior

Group	Behavior	Pre-Treatment Median	$25^{th} - 75^{th}$ Percentile	Post-Treatment Median	$25^{th} - 75^{th}$ Percentile
Desensitization	Cringing	.052/min	.008 - .096	.014/min	..002 - .038
Husbandry	Cringing	.014/min	.004 - .112	.010/min	.004 - .071
Control	Cringing	.033/min	.008 – .121	.065/min	.011 - .082
Desensitization	Stress-Related	.221/min	.110 - .313	.116/min	.077 - .190
Husbandry	Stress-Related	.239/min	.125 - .432	.179/min	.128 - .328
Control	Stress-Related	.192/min	.116 - .354	.131/min	.101 - .236
Desensitization	Cringing/humans	.026/min	.006 - .043	.007/min	.000 - .016
Husbandry	Cringing/humans	.003/min	.000 - .026	.001/min	.000 - .014
Control	Cringing/humans	.023/min	.003 - .048	.015/min	.003 - .051

Table Three: Wilcoxon Matched-Pairs Sign Tests for Rates of Behavior: Desensitization Group

Behavior	Frequencies		Exact Significance
	Category	N	
Cringing toward Person	Negative Differences[a]	6	.016 (one-tailed)*
	Positive Differences[b]	0	
	Ties[c]	0	
Cringing (total)	Negative Differences[a]	6	.016 (one-tailed)*
	Positive Differences[b]	0	
	Ties[c]	0	
Stress Related	Negative Differences[a]	6	.016 (one-tailed)*
	Positive Differences[b]	0	
	Ties[c]	0	

* = significant at $p < .05$
[a] = pre-treatment median > post-treatment median
[b] = pre-treatment median < post-treatment median
[c] = pre-treatment median = post-treatment median

Table Four: Wilcoxon Matched-Pairs Sign Tests for Rates of Behavior: Husbandry Group

Behavior	Frequencies		Exact Significance
	Category	N	
Cringing toward Person	Negative Differences[a]	3	.500 (one-tailed)
	Positive Differences[b]	2	
	Ties[c]	1	
Cringing (total)	Negative Differences[a]	3	.500 (one-tailed)
	Positive Differences[b]	3	
	Ties[c]	0	
Stress Related	Negative Differences[a]	5	.109 (one-tailed)
	Positive Differences[b]	1	
	Ties[c]	0	

* = significant at $p < .05$
[a] = pre-treatment median > post-treatment median
[b] = pre-treatment median < post-treatment median
[c] = pre-treatment median = post-treatment median

<u>**Table Five:** Wilcoxon Matched-Pairs Sign Tests for Rates of Behavior: Habituation Group</u>

Behavior	Frequencies		Exact Significance
	Category	N	
Cringing toward Person	Negative Differences[a]	3	.500 (one-tailed)
	Positive Differences[b]	2	
	Ties[c]	1	
Cringing (total)	Negative Differences[a]	2	.344 (one-tailed)
	Positive Differences[b]	4	
	Ties[c]	0	
Stress Related	Negative Differences[a]	5	.109 (one-tailed)
	Positive Differences[b]	1	
	Ties[c]	0	

* = significant at $p < .05$
[a] = pre-treatment median > post-treatment median
[b] = pre-treatment median < post-treatment median
[c] = pre-treatment median = post-treatment median

We computed difference scores for each subject as the average rate of a behavior in the pre-treatment phase minus the average rate of that behavior in the post-treatment phase. Using these difference scores, a Kruskal-Wallis test was run to determine if there was a significant difference between the three treatment groups regarding these difference scores (Table 6). This test indicated that there was not a significant effect, overall, of group membership on the difference scores in terms of rates of behavior (Figures 4 – 6).

Table Six: Kruskal-Wallis Test for Group Effects on Difference Scores (Rates of Behavior)

Behavior	Chi-Square	Degrees of Freedom	P Value
Cringe (total)	2.570	2	.277 (two-tailed)
Stress Related	.421	2	.810 (two-tailed)
Cringe toward Person	3.597	2	.166 (two-tailed)

Figure Four: Difference Scores for Rate of Cringing Behavior (total)

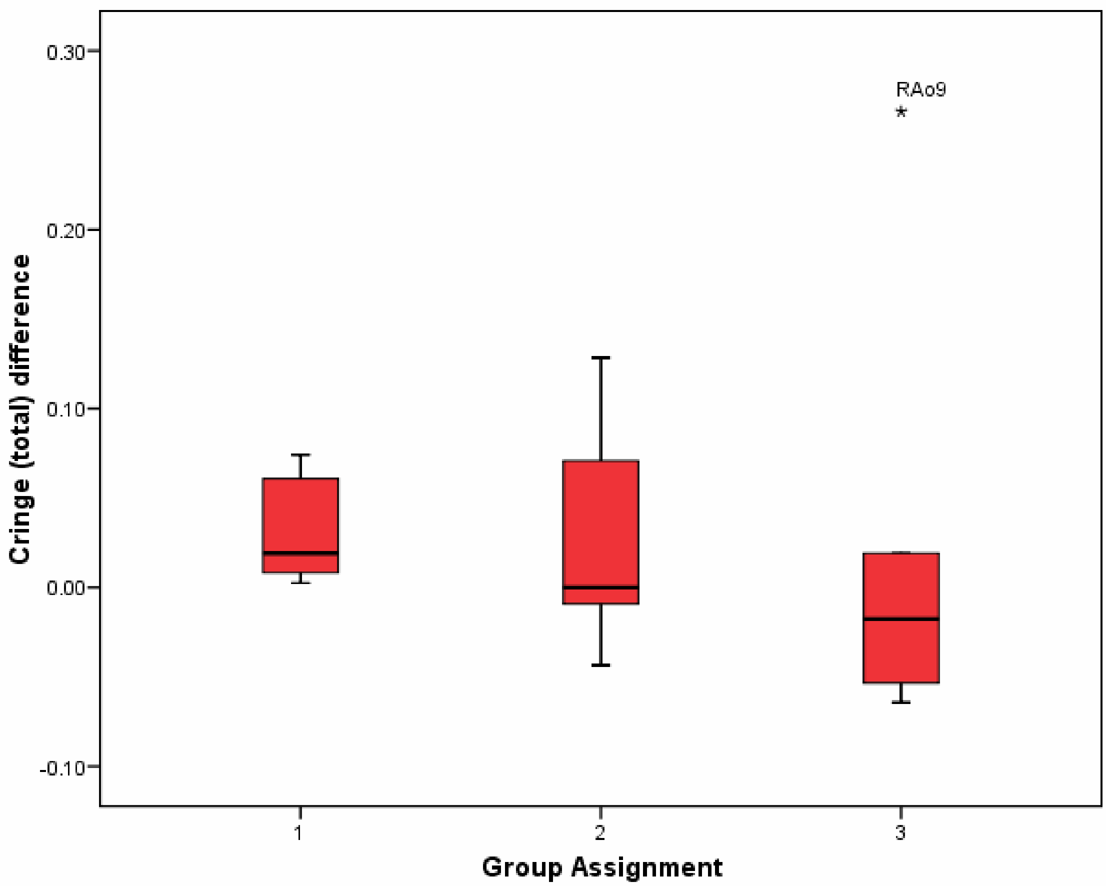


Figure Five: Difference Scores for Rate of Stress-Related Behavior

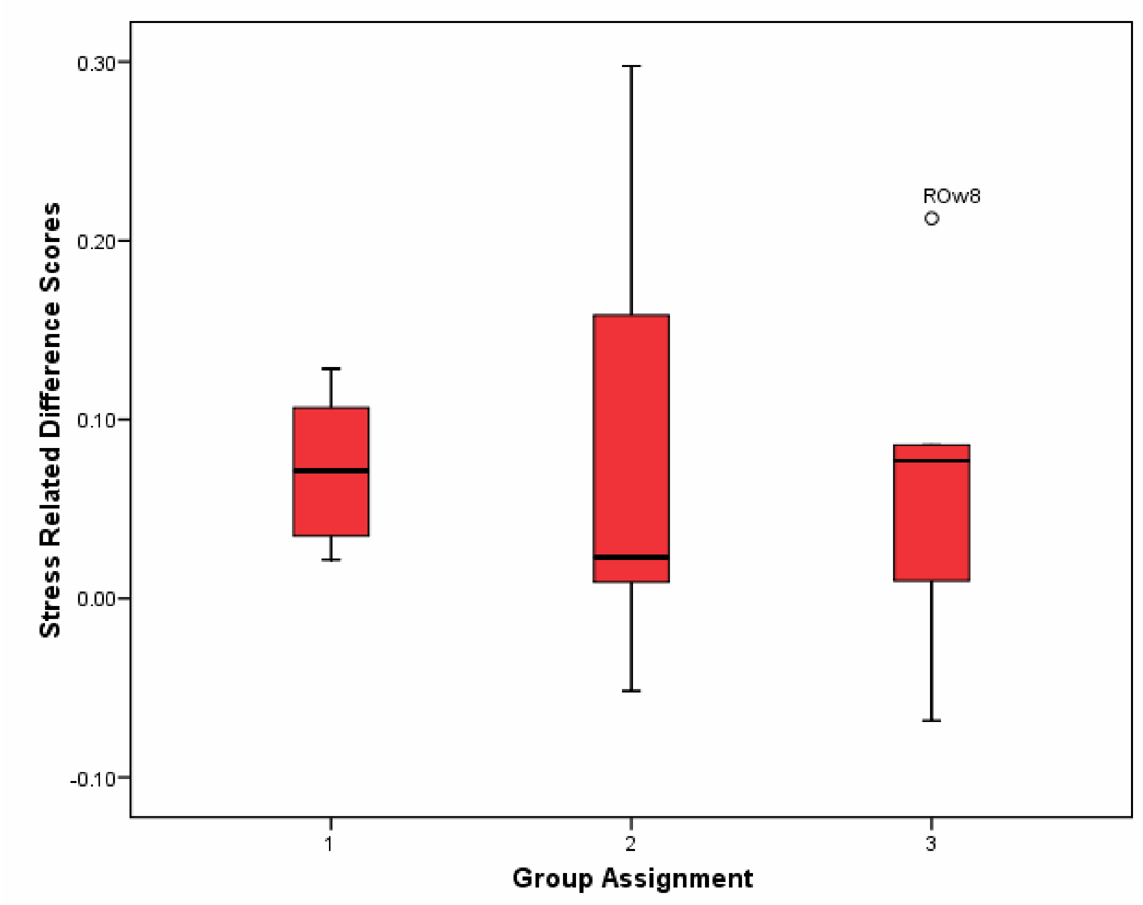
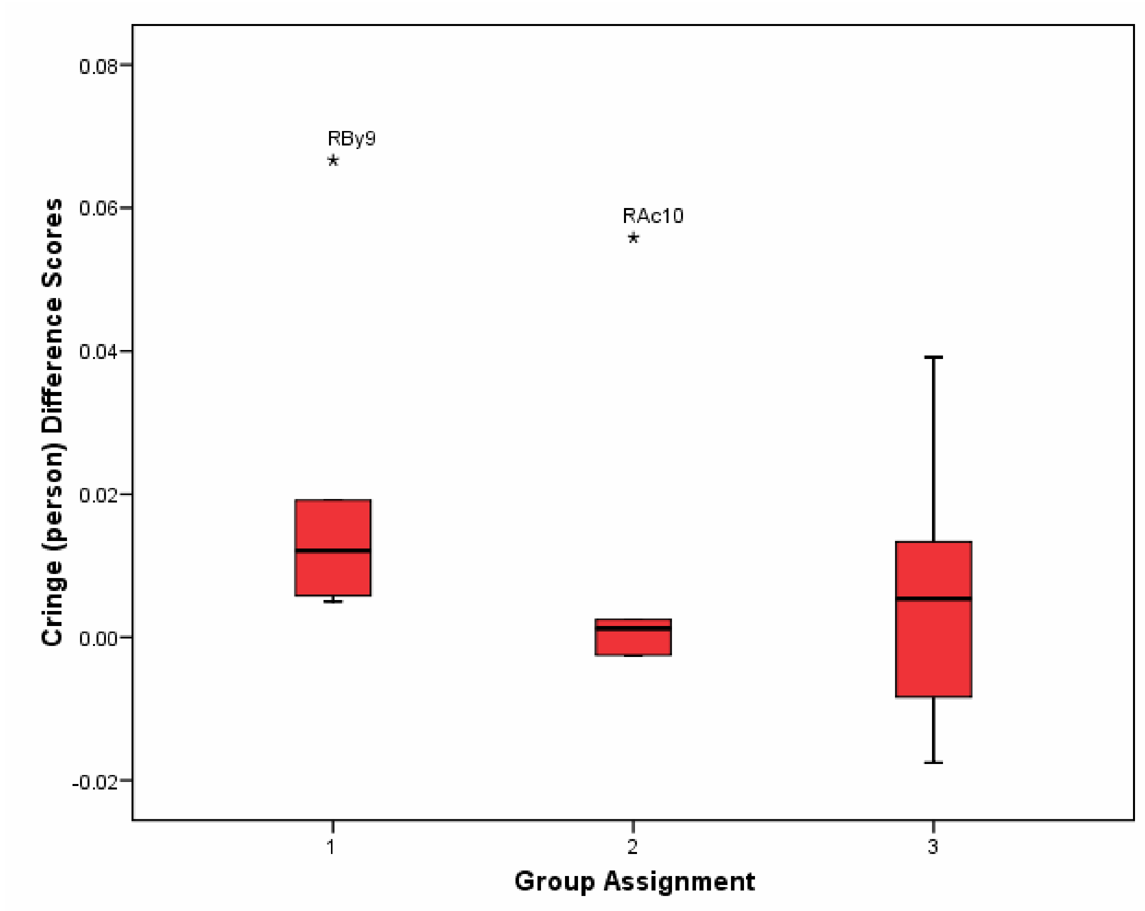


Figure Six: Difference Scores for Rate of Cringing toward Humans



Finally, in our collection of behavioral data we recorded instances of the following events: 1) a person enters the room and approaches the cage; 2) a person enters the room and sprays a hose near the cage; 3) a person enters the room and inserts a chow stick into the cage; 4) a person enters the room and adds some item to the cage (food or enrichment); 5) a loud noise occurs. The first four of these events were grouped into a "human activity" fear trigger category, and loud noise was considered alone. These "triggers" were analyzed for correlations between rates of the two triggers and rates of cringing behavior (total), stress-related behaviors, and cringing behavior directed toward humans. Strong correlations were found between the rate of loud noise and the rate of stress-related behavior in both Phase 1 of the study and Phase 2, $r = .676$, $n = 18$, $p < .01$, $r = .659$, $n = 18$, $p < .01$). Correlations between loud noise and either subset of cringing behaviors, between human activity triggers and stress behaviors, and between human activity triggers and either subset of cringing behaviors were not strong in either phase of the study (see Table 7).

Table Seven: Pearson's Correlations between Rates of Behavior and Rates of Fear Triggers (N = 18)

Behavior/Trigger	Human Activity Phase One		Loud Noise Phase One		Human Activity Phase Two		Loud Noise Phase Two	
	r	p	r	p	r	p	r	p
Cringe Phase One	.025	.922	-.305	.219	NA		NA	
Stress Phase One	-.085	.738	.676	.002**	NA		NA	
Cringe/human Phase One	.094	.710	-.276	.267	NA		NA	
Cringe Phase Two	NA		NA		.391	.109	-.172	.494
Stress Phase Two	NA		NA		-.084	.740	.659	.003**
Cringe/person Phase Two	NA		NA		.365	.136	-.301	.225

** = p < .01

Duration of Behavior Analyses

Duration of behavior was analyzed in terms of the percent of each observation (half-hour intervals) dedicated to a certain activity. Categories formed for purpose of analyses consisted of: 1) Cringing (cringing, freezing); 2) Affiliative behaviors (lip smack, coo, affiliative contact); 3) Aggressive (cage shake, ear flick, open mouth stare, crook tail); 4) Stress (yawn, body shake, scratch); 5) Self-Directed behaviors (bite nails, lick self, self-groom, self play); 6) Nonsocial behaviors (eat, inactive, locomote, manipulate object, huddle, scan); 7) Abnormal behaviors (urine drink, masturbate, deposit food, self clasp, bizarre posture, eye poke, floating limb, self-mouth, self-slap, self-injurious behavior, flipping, over-groom, pacing, head toss, rocking). Separately, cringing as directed toward a person was assessed, and also separately, time spent in the back of the cage was assessed.

A Wilcoxon Matched-Pairs Sign Test was conducted to assess the direction of change for each treatment group regarding cringing as directed towards humans, cringing in total, stress-related behaviors, and time spent in the back of the cage. This test revealed a significant number of subjects in the desensitization group showing a decrease in cringing as directed toward humans (exact significance = .016, one-tailed, N – ties = 6) (Figure 9), but not a significant number of subjects exhibiting a decrease in cringing in total (exact significance = .109, one-tailed, N – ties = 6) (Figure 8) or in stress-related behaviors (exact significance = .109, N – ties = 6) (Figure 7, Table 9). Neither of the other two treatment groups showed a significant number of subjects with a decrease in these behaviors (Figure 7 – 9, Table 10 – 11). For medians and percentiles, see Table 8.

Figure Seven: Duration of Stress-Related Behaviors

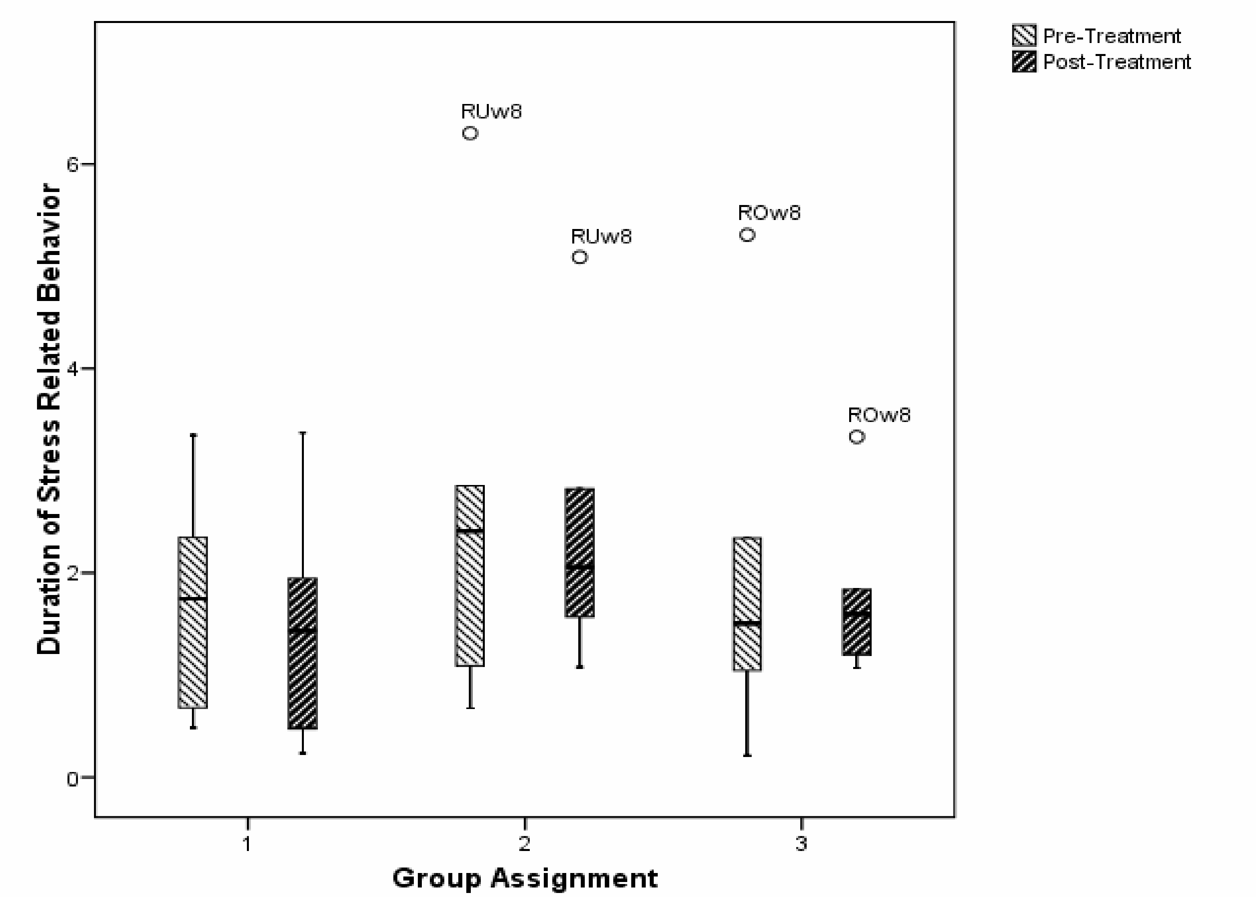


Figure Eight: Duration of Cringing Behaviors

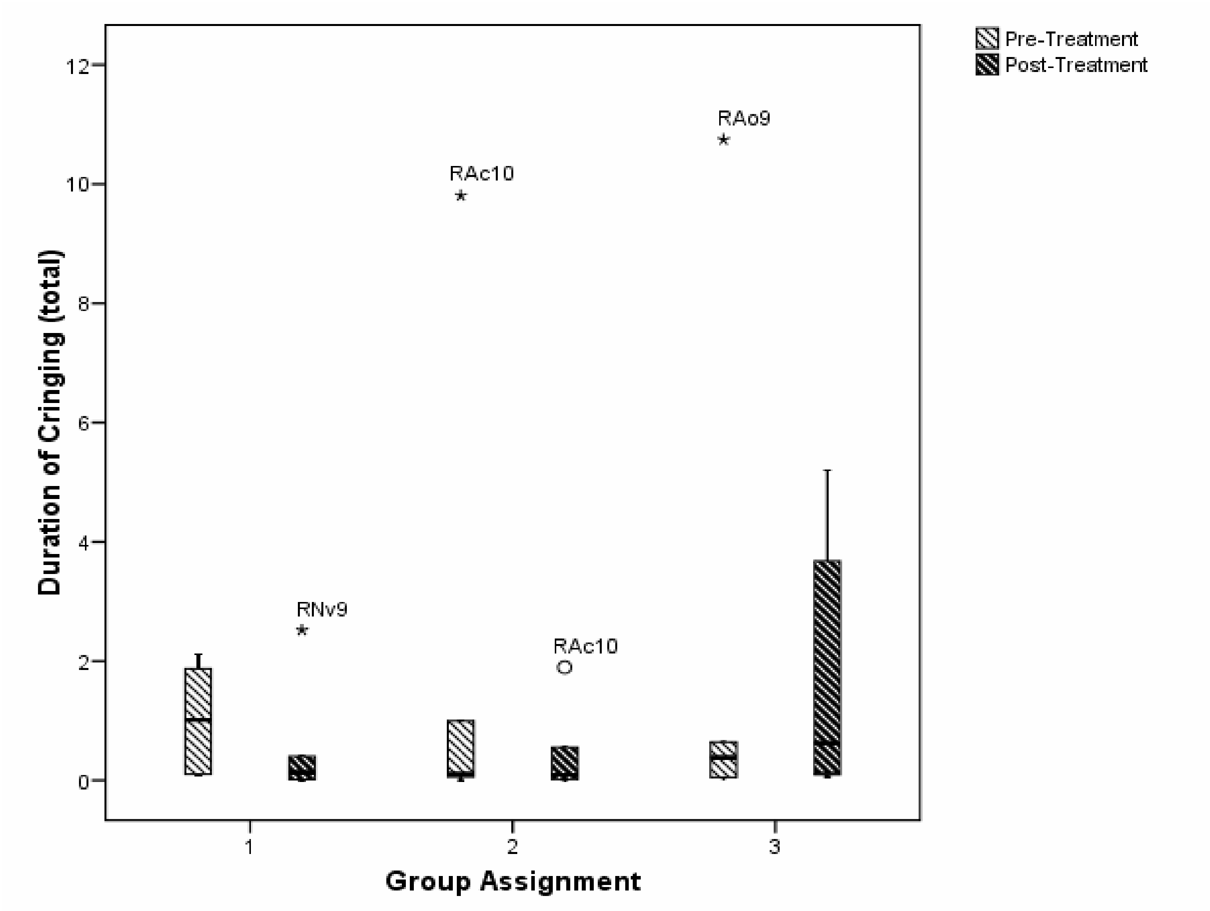


Figure Nine: Duration of Cringing toward Humans

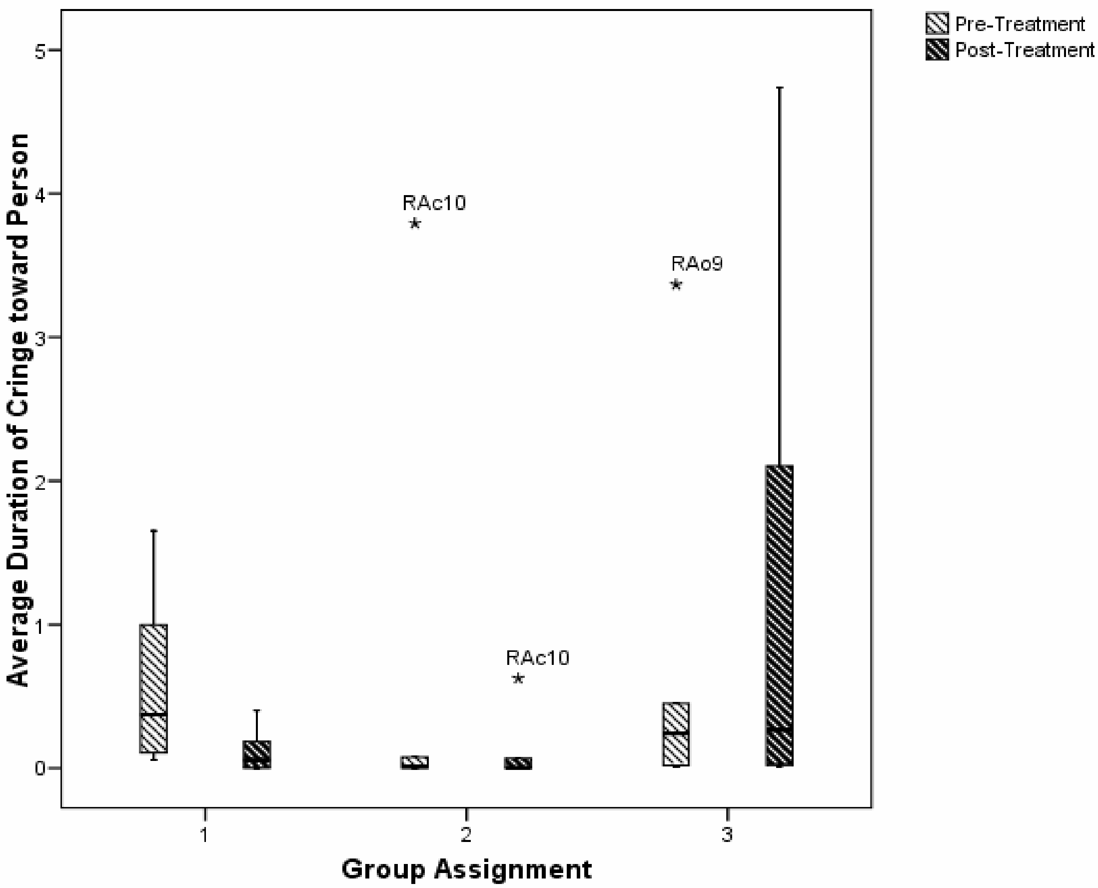


Table Eight: Medians and Percentiles for Durations of Behavior

Group	Behavior	Pre-Treatment Median	$25^{th} – 75^{th}$ Percentile	Post-Treatment Median	$25^{th} – 75^{th}$ Percentile
Desensitization	Cringing	1.012 %	.100 – 1.933	.136 %	.013 - .932
Husbandry	Cringing	.107 %	.046 – 3.200	.095 %	.018 - .891
Control	Cringing	.385 %	.050 – 3.169	.628 %	.090 – 4.057
Desensitization	Stress-Related	1.748 %	.632 – 2.598	1.435 %	.415 – 2.303
Husbandry	Stress-Related	2.409 %	.989 – 3.715	2.057 %	1.446 – 3.389
Control	Stress-Related	1.508 %	.838 – 3.082	1.597 %	1.169 – 2.213
Desensitization	Cringing/humans	.372 %	.095 – 1.161	.057 %	.000 - .239
Husbandry	Cringing/humans	.012 %	.000 – 1.006	.003 %	.000 - .206
Control	Cringing/humans	.243 %	.018 – 1.179	.266 %	.019 – 2.763
Desensitization	Back of Cage	38.748%	11.310 – 51.726	29.321 %	8.997 – 47.133
Husbandry	Back of Cage	20.372 %	12.309 – 25.547	14.170 %	9.731 – 17.525
Control	Back of Cage	20.438 %	15.463 – 48.883	28.511 %	20.945 – 55.601

Table Nine: Wilcoxon Matched-Pairs Sign Tests for Durations of Behavior: Desensitization Group

Behavior	Frequencies		Exact Significance
	Category	N	
Cringing toward Person	Negative Differences[a]	6	.016 (one-tailed)*
	Positive Differences[b]	0	
	Ties[c]	0	
Cringing (total)	Negative Differences[a]	5	.109 (one-tailed)
	Positive Differences[b]	1	
	Ties[c]	0	
Stress Related	Negative Differences[a]	5	.109 (one-tailed)
	Positive Differences[b]	1	
	Ties[c]	0	

* = significant at $p < .05$
[a] = pre-treatment median > post-treatment median
[b] = pre-treatment median < post-treatment median
[c] = pre-treatment median = post-treatment median

Table Ten: Wilcoxon Matched-Pairs Sign Tests for Durations of Behavior: Husbandry Group

Behavior	Frequencies		Exact Significance
	Category	N	
Cringing toward Person	Negative Differences[a]	3	.500 (one-tailed)
	Positive Differences[b]	2	
	Ties[c]	1	
Cringing (total)	Negative Differences[a]	2	.344 (one-tailed)
	Positive Differences[b]	4	
	Ties[c]	0	
Stress Related	Negative Differences[a]	3	.500 (one-tailed)
	Positive Differences[b]	3	
	Ties[c]	0	

* = significant at $p < .05$
[a] = pre-treatment median > post-treatment median
[b] = pre-treatment median < post-treatment median
[c] = pre-treatment median = post-treatment median

Table Eleven: Wilcoxon Matched-Pairs Sign Tests for Durations of Behavior: Habituation Group

Behavior	Frequencies		Exact Significance
	Category	N	
Cringing toward Person	Negative Differences[a]	3	.500 (one-tailed)
	Positive Differences[b]	3	
	Ties[c]	0	
Cringing (total)	Negative Differences[a]	2	.344 (one-tailed)
	Positive Differences[b]	4	
	Ties[c]	0	
Stress Related	Negative Differences[a]	3	.500 (one-tailed)
	Positive Differences[b]	3	
	Ties[c]	0	

* = significant at $p < .05$
[a] = pre-treatment median > post-treatment median
[b] = pre-treatment median < post-treatment median
[c] = pre-treatment median = post-treatment median

The average percent of time each subject spent in the back of the cage was also analyzed. A Wilcoxon Matched-Pairs Sign Test did not indicate a significant number of subjects with a decrease in this percentage for any of the treatment groups (for all groups, exact significance = .219, N – ties = 6) (Figure 10, Table 12).

Figure Ten: Percent of Interval Spent in Back of Cage

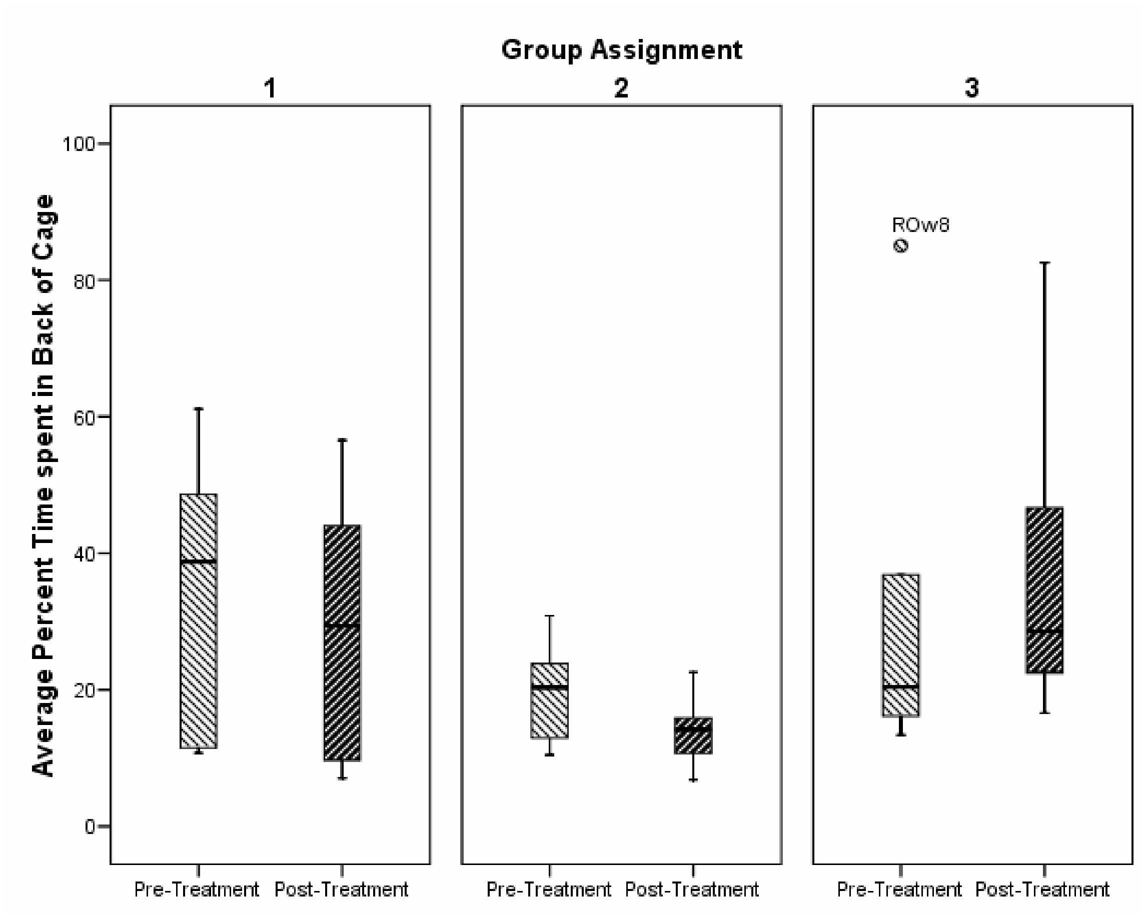


Table Twelve: Wilcoxon Matched-Pairs Sign Tests for Durations of Behavior: Time in Back of Cage

Group	Frequencies		Exact Significance
	Category	N	
Desensitization	Negative Differences[a]	5	.219 (two-tailed)
	Positive Differences[b]	1	
	Ties[c]	0	
Husbandry	Negative Differences[a]	5	.219 (two-tailed)
	Positive Differences[b]	1	
	Ties[c]	0	
Habituation	Negative Differences[a]	1	.219 (two-tailed)
	Positive Differences[b]	5	
	Ties[c]	0	

* = significant at p < .05
[a] = pre-treatment median > post-treatment median
[b] = pre-treatment median < post-treatment median
[c] = pre-treatment median = post-treatment median

Difference scores for the different behaviors and for the subjects' location in the cage were calculated as the average duration of time engaged in that behavior or location during pre-treatment minus the average durations for that behavior or location during post-treatment. A Kruskal Wallis test comparing these difference scores between groups found a significant effect of group membership on the change in duration of time spent in the back of the cage ($\chi^2 = 6.42$, df = 2, $p < .05$, two-tailed) (Figure 11) and a significant effect of group membership on the change in duration of time spent cringing toward people ($\chi^2 = 7.73$, df = 2, $p < .05$, two-tailed) (Figure 12). There was not a significant effect of group on change in the duration of either stress-related behaviors (Figure 13) or cringing behaviors in total (Figure 14, Table 13).

Figure Eleven: Difference Scores for Duration of Time in Back of Cage

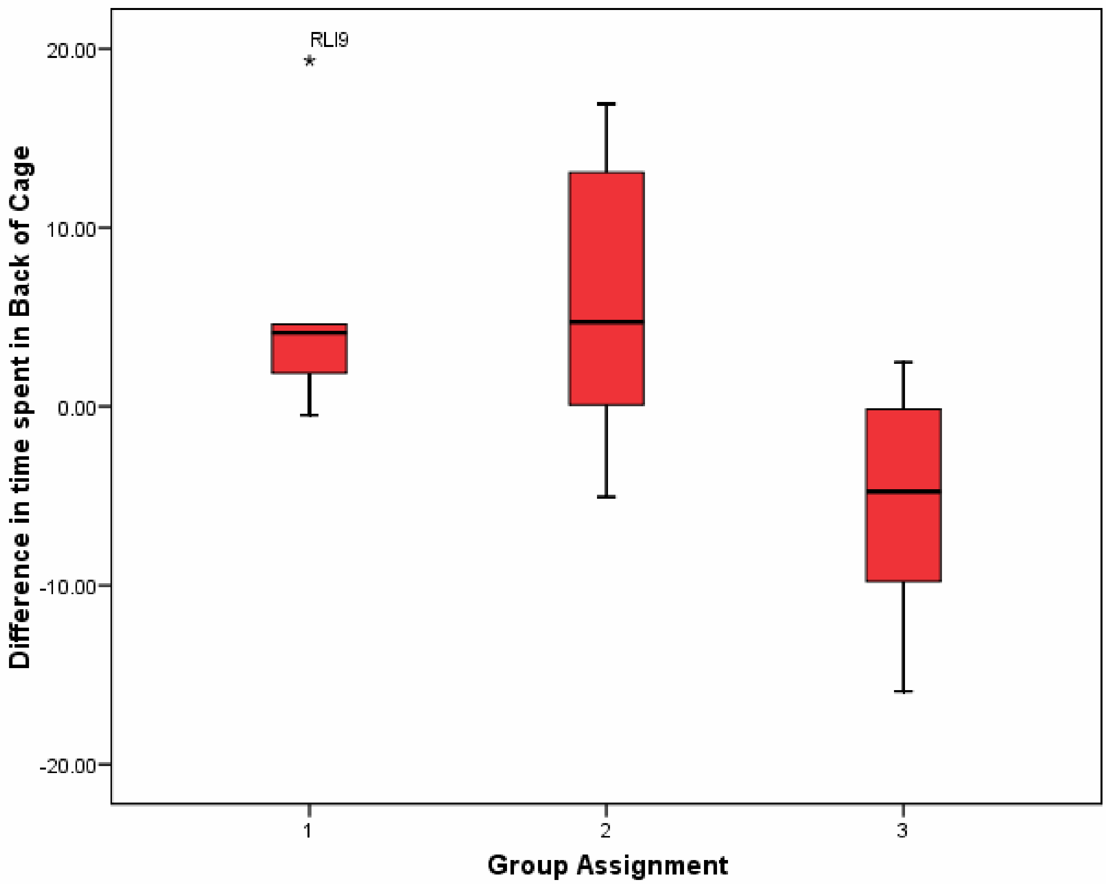


Figure Twelve: Difference Scores for Duration of Time spent Cringing toward Humans

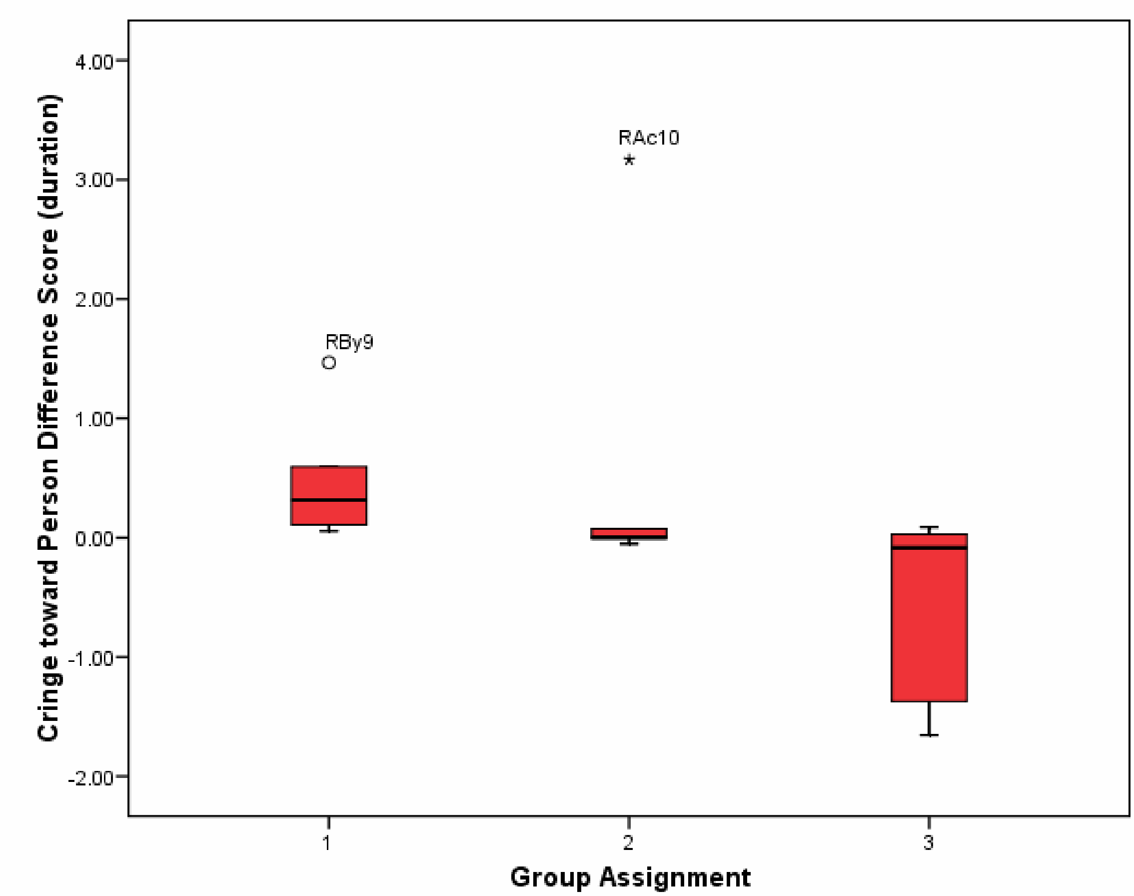


Figure Thirteen: Difference Scores for Duration of Time of Stress-Related Behaviors

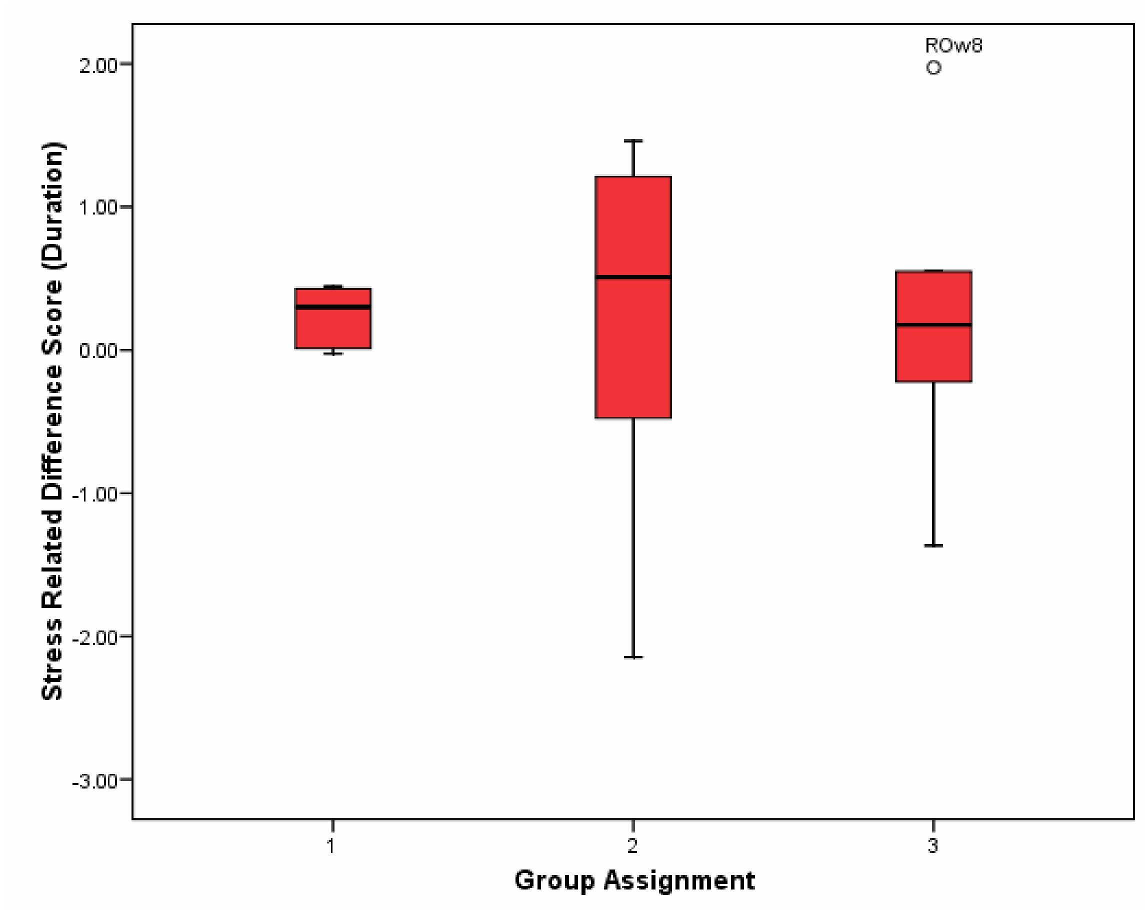


Figure Fourteen: Difference Scores for Duration of Time Cringing (total)

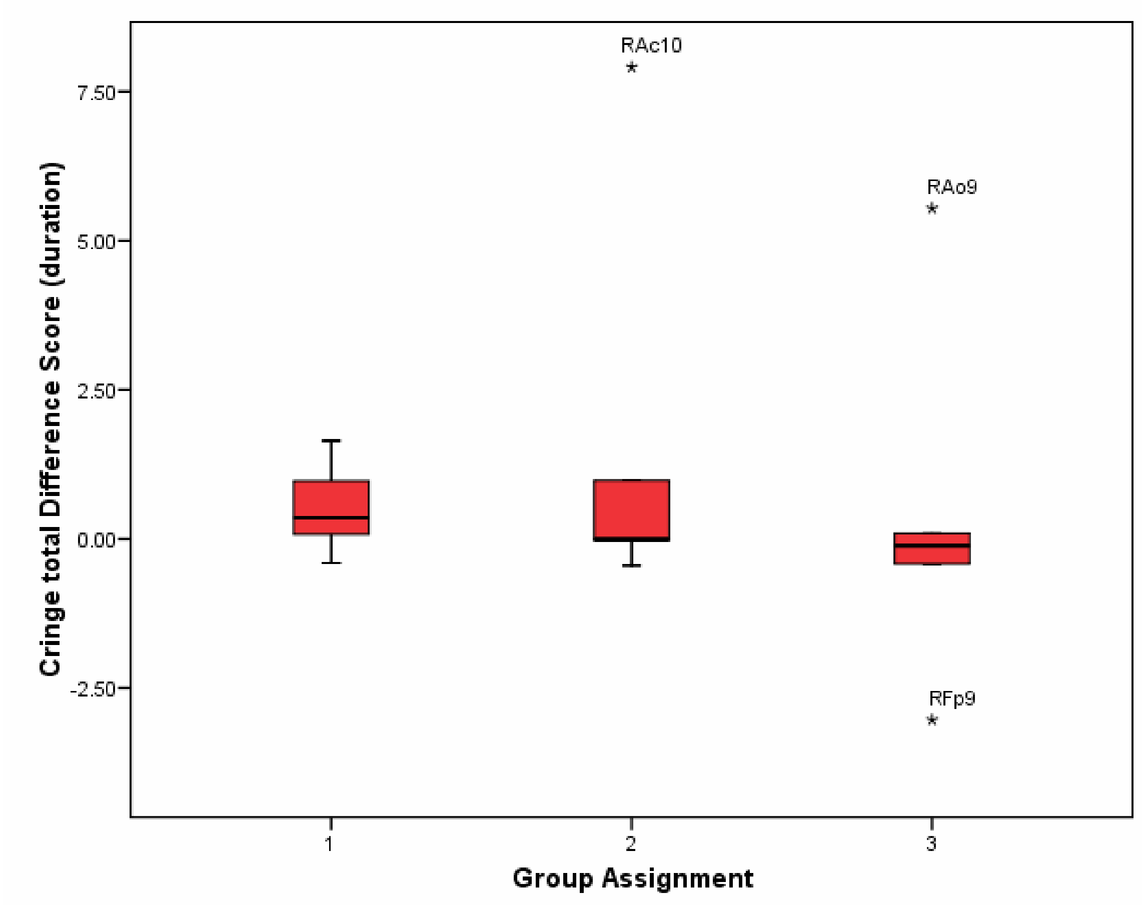


Table Thirteen: Kruskal-Wallis Test for Group Effects on Difference Scores (Durations of Behavior)

Behavior	Chi-Square	Degrees of Freedom	P Value
Cringe (total)	1.719	2	.423 (two-tailed)
Stress Related	.035	2	.983 (two-tailed)
Cringe toward Person	7.730	2	.021 (two-tailed)*
Time in Back of Cage	6.421	2	.040 (two-tailed)*

* significant at $p < .05$

Temperament Analyses

Temperament Test scores were evaluated for each animal by assigning Likert scale numeric scores to various behavioral responses to parts of the test. The following responses received these rating scores: 1) Initial Response to Observer; 2) Predominant Response to Observer 3) Initial Response to Direct Eye Contact 4) Predominant Response to Direct Eye Contact 5) Initial Response to Novel Food 6) Initial Response to Novel Object. Ratings were ordinal from 1 – 5 where 1 was the least fearful and 5 was the most fearful. Latency times (in seconds) were recorded for each animal's response to the novel food and novel object. Responses included inspection of the food or object (sniffing, peering at), touching the food or object, manipulation of the food or object, and for the food, putting the food item in the mouth. Based on these latency scores, the following ratings were applied, based on ratings used by Coleman, et al. (2005): 1 = less than 5 seconds (exploratory); 2 = greater than 5 seconds (moderate); 3 = never did the behavior (inhibited).

Once each response had been assigned a rating, behavioral responses were averaged for each test across the following categories: 1) Average response to Observer; 2) Initial Response to Novel Food; 3) Average latency rating in Response to Novel Food; 4) Initial Response to Novel Object; 5) Average latency rating in Response to Novel Object. Based on these average ratings, a Wilcoxon Matched-Pairs Signed-Ranks Test was run to assess the stability of each category of scores for the subjects as a group, and the results indicated that ratings did not differ significantly from Test 1 to Test 2 (see Figure 15, Table 14). Additionally, categories were collapsed into one average score for Test 1 and Test 2, and these scores were also shown not to differ significantly (Table 14).

Figure Fifteen: Temperament Score Stability for Individuals from Test One (pre-treatment) to Test Two (post-treatment)

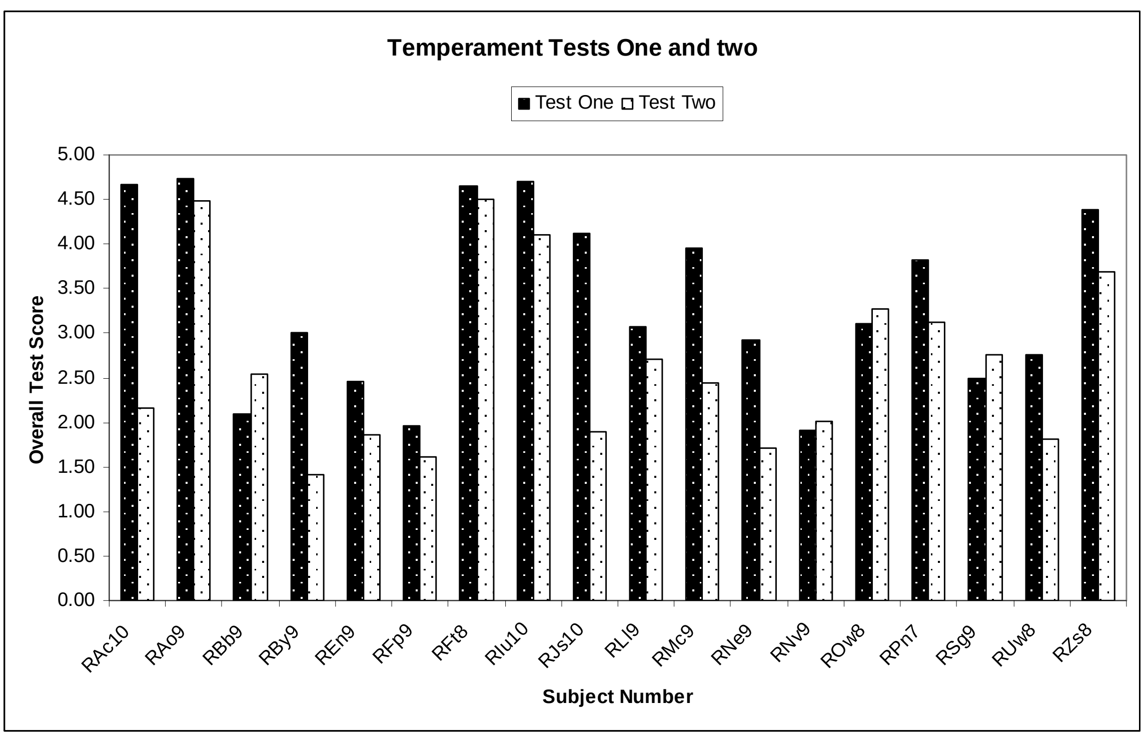


Table Fourteen: Wilcoxon Matched-Pairs Signed Ranks Test Assessment of Stability of Ratings for all subjects between Temperament Test One (pre-treatment) and Test Two (post-treatment)

Test Response	N	Z scores	p value (two-tailed)
Average Response to Observer	18	-.087	.930
Initial Response to Novel Food	18	-.766	.443
Average Response to Novel Food	18	-.328	.743
Initial Response to Novel Object	18	-.022	.982
Average Response to Novel Object	18	-.633	.527
Temperament Test One – Test Two	18	-.392	.695

We then transformed average scores for Test 1 and Test 2 into rank orders, Temperament Rank One and Temperament Rank Two. These ranks reflected each subject's temperament ratings relative to all other subjects both pre-treatment and post treatment. These two rank orders were then compared to each other with a Wilcoxon Matched-Pairs Signed-Ranks Test. The rankings were not significantly different from Test One to Test Two (z = -.392, n = 18, p = .695).

Ultimately, Temperament Scores were assigned to each animal based only on the first tests' average latency responses (in seconds), which were already determined to be stable in previous Signed-Rank Tests and which were the only measures used to determine temperament in previous studies using this same temperament assessment format (Coleman, et al., 2005). Each aspect of the novel object test (inspecting, touching, and manipulating the object) had a score associated with it that represented the number of seconds until the animal performed that response. If an animal never did a certain behavior, that animal was assigned a score of 500 seconds for that aspect of the test (more than the amount of time an animal had to engage the object, which was 5 minutes, or 300 seconds). Animals with an average latency score over 300 seconds were considered inhibited. Animals that scored between 5 – 300 seconds were considered moderate. Animals that scored below 5 seconds were considered exploratory (see Table 15).

Table Fifteen: Subject's Average Latency scores for Novel Objects and Temperament Assignments

Animal	Group	Latency	Category
RAc10	2	375	inhibited
RAo9	3	315.5	inhibited
RBb9	1	5.75	moderate
RBy9	1	2.75	exploratory
REn9	3	4	exploratory
RFp9	3	0	exploratory
RFt8	3	375	inhibited
RIu10	3	176.25	moderate
RJs10	2	5.5	moderate
RLl9	1	6.25	moderate
RMc9	1	48.5	moderate
RNe9	2	104.75	moderate
RNv9	1	0	exploratory
ROw8	3	7.5	moderate
RPn7	2	134.25	moderate
RSg9	2	2.5	exploratory
RUw8	2	3	exploratory
RZs8	1	323	inhibited

Response Acquisition Rates

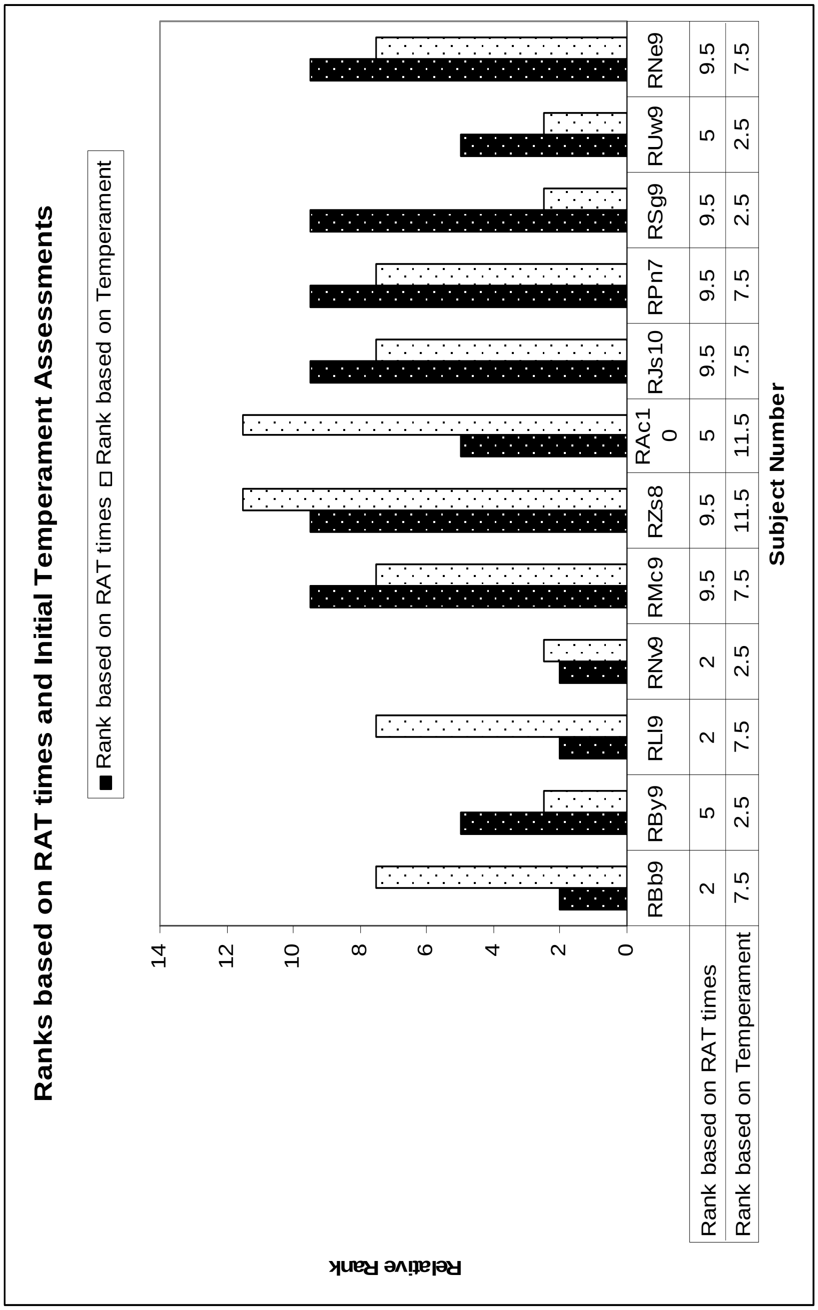
Animals that were assigned to either the desensitization or husbandry group were trained in 5 minute sessions for 25 sessions on various behaviors (see Appendix D for behaviors and shaping steps for each behavior). During each 5 minute training session, the pre-defined steps of each behavior's shaping program that were reached were recorded. For each step of each behavior, the number of sessions (at 5 minutes each) required to reach that step was recorded. To be considered achieved, a certain step had to be met for two 5 minute sessions in a row. The time required for each step of each behavior was calculated as the number of minutes required to reach that step. Therefore, if Step 1 required 4 sessions, the acquisition time for that step would be 20 minutes. If 2 steps were reached within a single session, then the amount of time required to get to that pair of steps would be divided by two. If an animal failed to reach the next step of a shaping program for a certain behavior, the last step was recorded as having taken 100 minutes, and no further steps were considered when calculating the final rate. Ultimately, the acquisition times for each step were summed and then divided by the number of steps total for each animal, resulting in a final Rate of Acquisition (RAT). The final range of RATs for the subjects in the training groups was from approximately 3 minutes per step to approximately 24 minutes per step. Animals were then categorized as slow, average, or fast learners based on their RAT scores. Animals with an RAT between 17 – 24 minutes were considered slow, those with an RAT of 9 – 16 were considered average, and those with an RAT of 1 – 8 were considered fast learners (see Table 16).

Table Sixteen: Rates of Acquisition for Animals in Groups One and Two (Desensitization and Training)

Animal Code	RAT	Speed of Learning	Initial Temperament
RBb9	3.86	fast	moderate
RBy9	9.09	average	exploratory
RLl9	5.46	fast	moderate
RNv9	2.05	fast	exploratory
RMc9	20.29	slow	moderate
RZs8	20.67	slow	inhibited
RAc10	13.50	average	inhibited
RJs10	21	slow	moderate
RPn7	17.50	slow	moderate
RSg9	19.64	slow	exploratory
RUw8	12.89	average	exploratory
RNe9	23.89	slow	moderate

We then assigned a value of 1 to fast learners, 2 to average learners, and 3 to slow learners. A value of 1 was also assigned to exploratory temperaments, 2 to moderate temperaments, and 3 to inhibited temperaments. A Wilcoxon Matched-Pairs Signed-Ranks Test was used to assess the difference in ratings, and indicated that the ratings were, overall, not significantly different from each other, $z = -1.387$, $p = .166$ (two-tailed) (Figure 16). Additionally, ratings were transformed into ranks, and a second Wilcoxon Test was run to determine if the animals that generally ranked as "slower" learners also ranked as more inhibited in terms of temperament. This test, also, was indicative of consistency between the two scores, $z = -.316$, $p = .752$ (two-tailed). However, correlation scores as assessed by a Spearman's rho between Temperament scores and RATs were not strong, $r_s = .460$, $n = 12$, $p = .460$ (two-tailed).

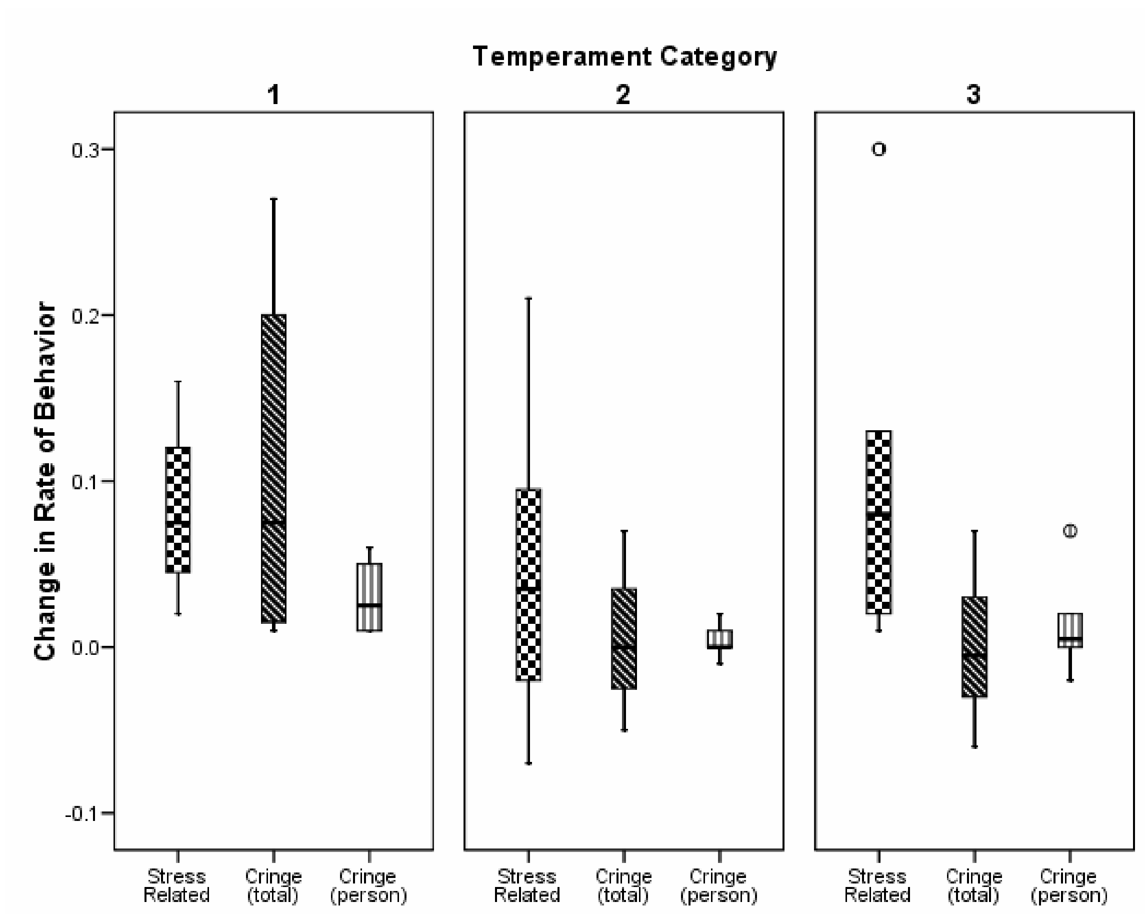
Figure Sixteen: Ranks of Animals in Groups One and Two Based on RAT and Initial Temperament



- Lower ranks = faster learners, more exploratory temperaments; higher ranks = slower learners, more inhibited temperaments

Since we had predicted that more inhibited animals would also not benefit as greatly from treatment during this study, a correlation between initial temperament scores and overall changes in rate of fear-related behaviors was tested using a Spearman's rho correlation test. No significant correlations were found in the direction of our hypothesis, though a near significant correlation in the reverse direction was found for temperament and cringing behavior difference scores (r_s = -.421, n = 18, p = .082 (two-tailed), indicating that, in this case, animals that were scored as having a more inhibited temperament at the start of treatment showed the biggest decrease in rate of cringing behavior as compared to animals of a more exploratory temperament (see Figure 17).

Figure Seventeen: Temperament Categories and Associated Difference Scores for Rates of Fear-Related Behaviors



1 = Exploratory 2 = Moderate 3 = Inhibited

These results were verified by a One-Way ANOVA testing for effects of Temperament Category on the average difference scores for each subject in regards to rates of stress-related behavior, cringing as directed toward humans, and overall cringing behaviors (see Tables 17 and 18). The only F-test approaching significance was for the effect of Temperament Category on rates of total cringing behavior, $F(2,15) = 3.612$, $p = .052$, and inspection of the means showed that this effect was in the opposite direction of our prediction (see Table 18, Figure 17). Similar tests were conducted to determine the effect of temperament on difference scores for durations of behavior. Results were much the same. A Spearman's rho indicated a significant correlation between temperament and duration of cringing behaviors ($r_s = -.525$, $p < .025$), again indicating an effect opposite to our prediction: animals with a more inhibited temperament rating showed more reduction in overall duration of cringing behavior than animals with a more exploratory temperament. This was again confirmed by running One-Way ANOVAs to test for effects of temperament category on difference scores for duration of behaviors. The only significant F value was for the effect of temperament on cringing behaviors, $F(2,15) = 5.393$, $p < .05$ (see Figure 18).

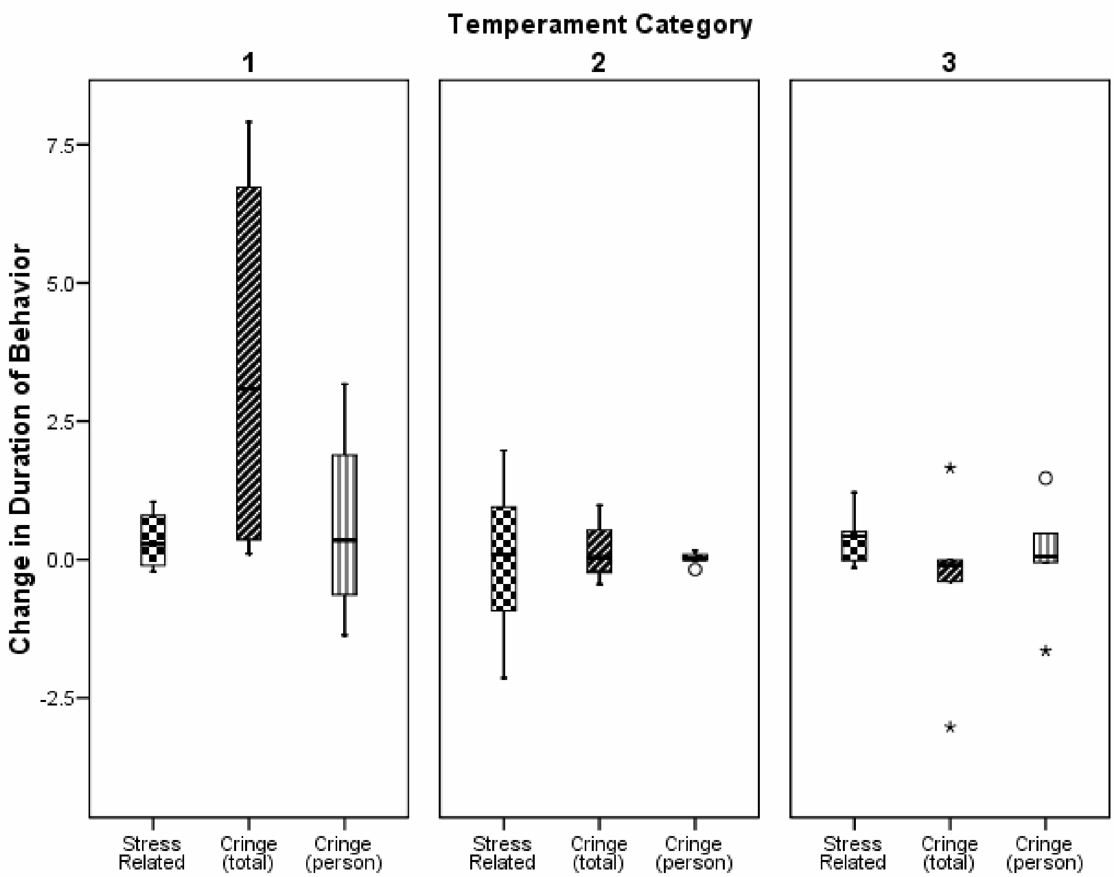
Table Seventeen: One Way ANOVA test of Effects of Temperament on Rates of Behavior

Difference Scores	Degrees of Freedom	F value	p value
Stress Behaviors	2,15	.735	.496
Cringing/human	2,15	2.151	.151
Cringing/total	2,15	3.612	.052

Table Eighteen: Means and Standard Deviations for Rates of Behaviors for the Different Temperament Categories

Difference Scores		N	Mean	Standard Deviation
Stress	Exploratory	6	.101	.106
	Moderate	8	.044	.899
	Inhibited	4	.084	.056
Cringe/person	Exploratory	6	.013	.029
	Moderate	8	.003	.008
	Inhibited	4	.029	.023
Cringe/total	Exploratory	6	.001	.048
	Moderate	8	.004	.044
	Inhibited	4	.106	.120

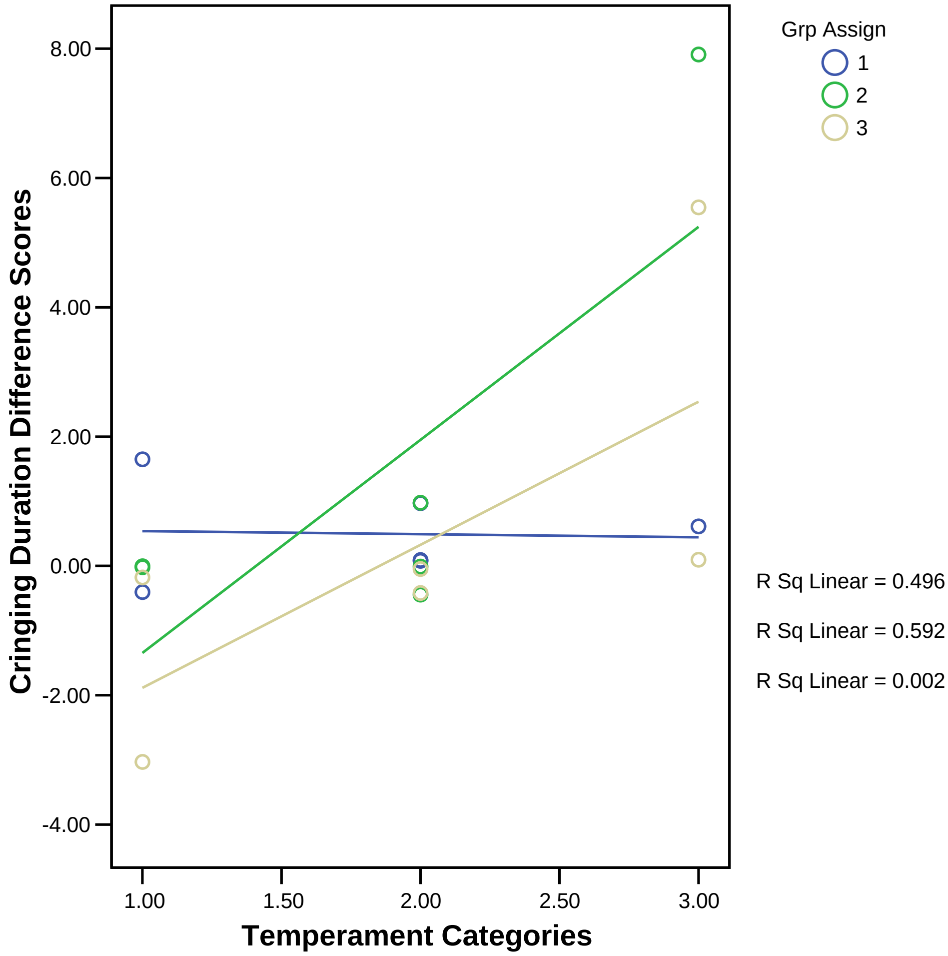
Figure Eighteen: Temperament Categories and Associated Difference Scores for Durations of Fear-Related Behaviors



1 = Exploratory   2 = Moderate   3 = Inhibited

One possibility was that there was an interaction effect between temperament and treatment groups. A scatter plot assessing the covariance of temperament and group assignment on difference scores for the duration of cringing showed a fairly strong linear relationship between these two variables in Groups 2 and 3 (Husbandry and Control), but not for Group 1 (Desensitization) (see Figure 19).

Figure Nineteen: Covariance between Temperament category and Group Assignment in effect on Difference scores for the Duration of Cringing Behavior



1 = Exploratory 2 = Moderate 3 = Inhibited

Response Test Analyses

One subject, RAo9, was dropped from the analysis of Response Test scores because of an error in the videotape for that animal which rendered the test impossible to score. For the rest of the subjects, scores were compared as a group for each part of the test (insertion of a chow stick to check the Lixit, insertion of a chow stick to remove excess chow, offer of a treat at the front of the cage, and spraying of a hose in front of the cage)

(Table 19). Additionally, an average score was calculated for each animal, and the group was tested for changes in average Response Test scores from Test 1 (pre-treatment) to Test 2 (post-treatment) (Figure 20). Comparisons were made using a Wilcoxon Matched-Pairs Signed-Ranks Test, and were followed up with similar tests on each of the treatment groups, independently. Results indicated a significant difference from Test 1 to Test 2 for all groups analyzed together on all aspects of the test other than the spraying of a hose, and also for average Response Test scores ($p < .05$) (Figure 21 – 24). When tests were run on the groups individually, however, the control group did not show a significant change in any measure. Both of the training groups (Group 1, desensitization, and Group 2, husbandry) had a lower rating on second tests for measures of response as averaged across all tests and on some particular measures of the test (see Table 20). Group 1 had significantly lower ratings for the Lixit test and the overall average test scores ($p < .05$), and Group 2 had significantly lower ratings for the Chow Removal Test, the Hose test, and the overall average test scores ($p < .05$).

Table Nineteen: Response Test Scores

Subject	Group	Lixit 1	Treat 1	Hose 1	Chow 1	Ave. T1	Lixit 2	Treat 2	Hose 2	Chow 2	Ave. T2
RAc10	2	5	6	3	5	4.75	3	6	3	3	3.75
RBb9	1	4	3	2	3	3	4	3	1	4	3
RBy9	1	4	6	3	4	4.25	4	1	3	3	2.75
REn9	3	4	3	3	4	3.5	3	1	3	3	2.5
RFp9	3	5	1	2	5	3.25	2	1	2	3	2
RFt8	3	3	6	3	4	4	3	6	1	4	3.5
RIu10	3	5	7	4	3	4.75	1	2	6	5	3.5
RJs10	2	4	1	3	4	3	2	1	1	2	1.5
RLl9	1	4	3	3	4	3.5	3	1	2	4	2.5
RMc9	1	4	6	4	4	4.5	3	2	3	2	2.5
RNe9	2	4	3	4	4	3.75	4	3	3	4	3.5
RNv9	1	5	1	3	5	3.5	4	1	1	3	2.25
ROw8	3	3	2	1	3	2.25	4	3	3	4	3.5
RPn7	2	3	3	4	4	3.5	3	1	1	2	1.75
RSg9	2	4	2	4	4	3.5	4	1	3	4	3
RUw8	2	3	1	5	4	3.25	3	3	4	4	3.5
RZs8	1	5	6	3	3	4.25	5	6	5	3	4.75

Figure Twenty: Average Response Test Scores for all Groups, Pre-Treatment and Post-Treatment

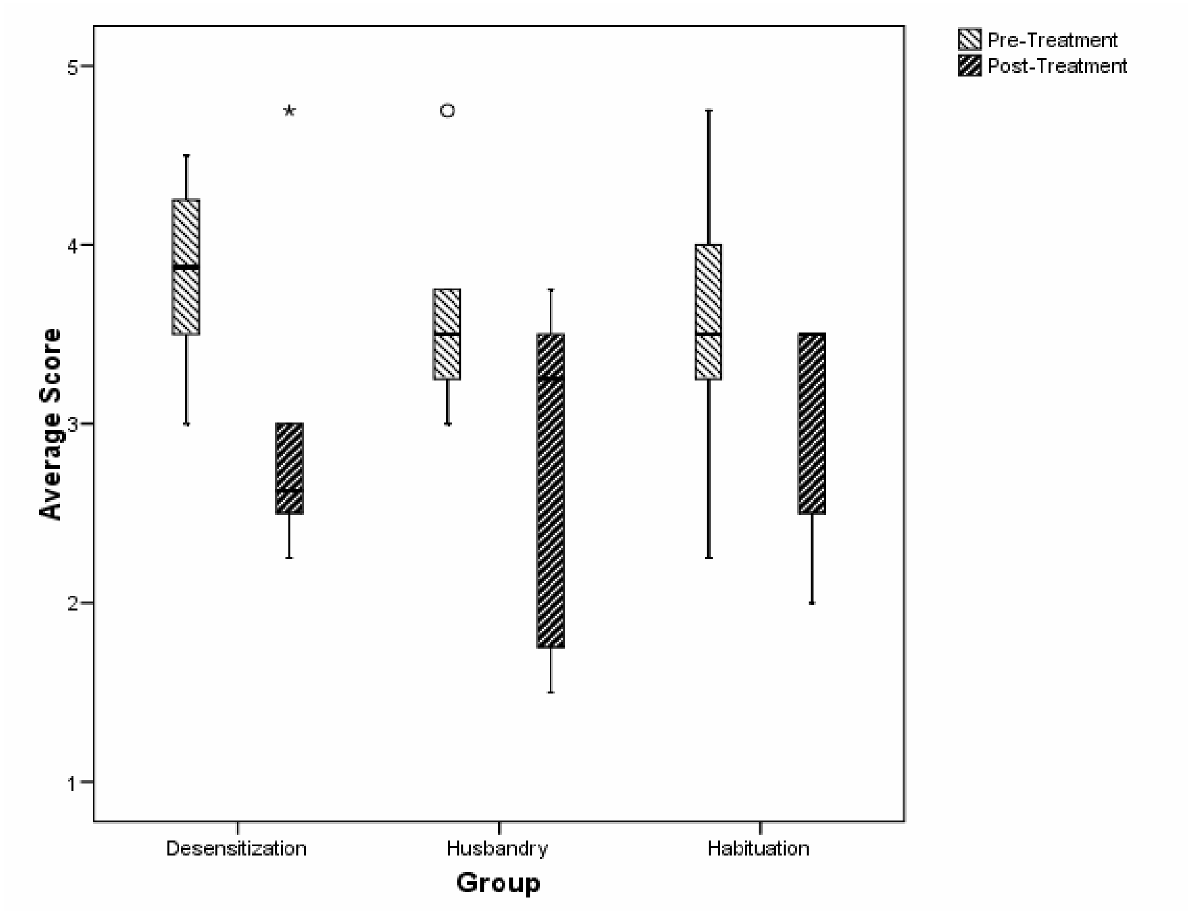


Table Twenty: Wilcoxon Matched-Pairs Signed-Rank Test Results for Changes in Response Test Scores (one-tailed)

	N	Lixit 1&2		Treat 1&2		Spray 1&2		Chow 1&2		Average 1&2	
		z	p	z	p	z	p	z	p	z	p
All Subs	18	-2.354	.010*	-1.976	.024*	-1.214	.113	-1.839	.033*	-2.698	.004**
Grp 1	6	-1.732	.042*	-1.604	.055	-.828	.204	-1.300	.097	-1.753	.040*
Grp 2	6	-1.414	.079	-.272	.393	-2.060	.020*	-1.732	.042*	-1.892	.029*
Grp 3	5	-1.289	.099	-1.069	.143	-.577	.282	.000	.500	-.962	.168

* = significant at p < .05
** = significant at p < .01

Figure Twenty-One: Response Test Scores for Checking the Lixit

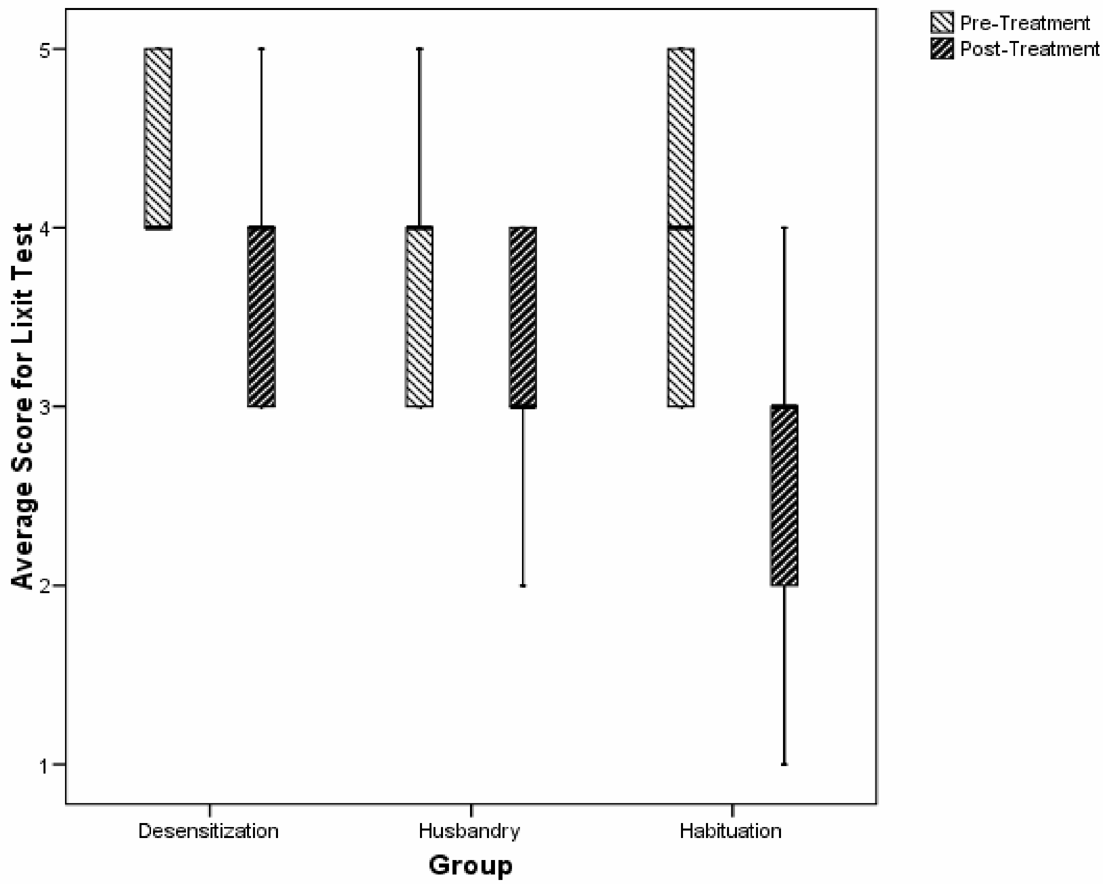


Figure Twenty-Two: Response Test Scores for Spraying a Hose

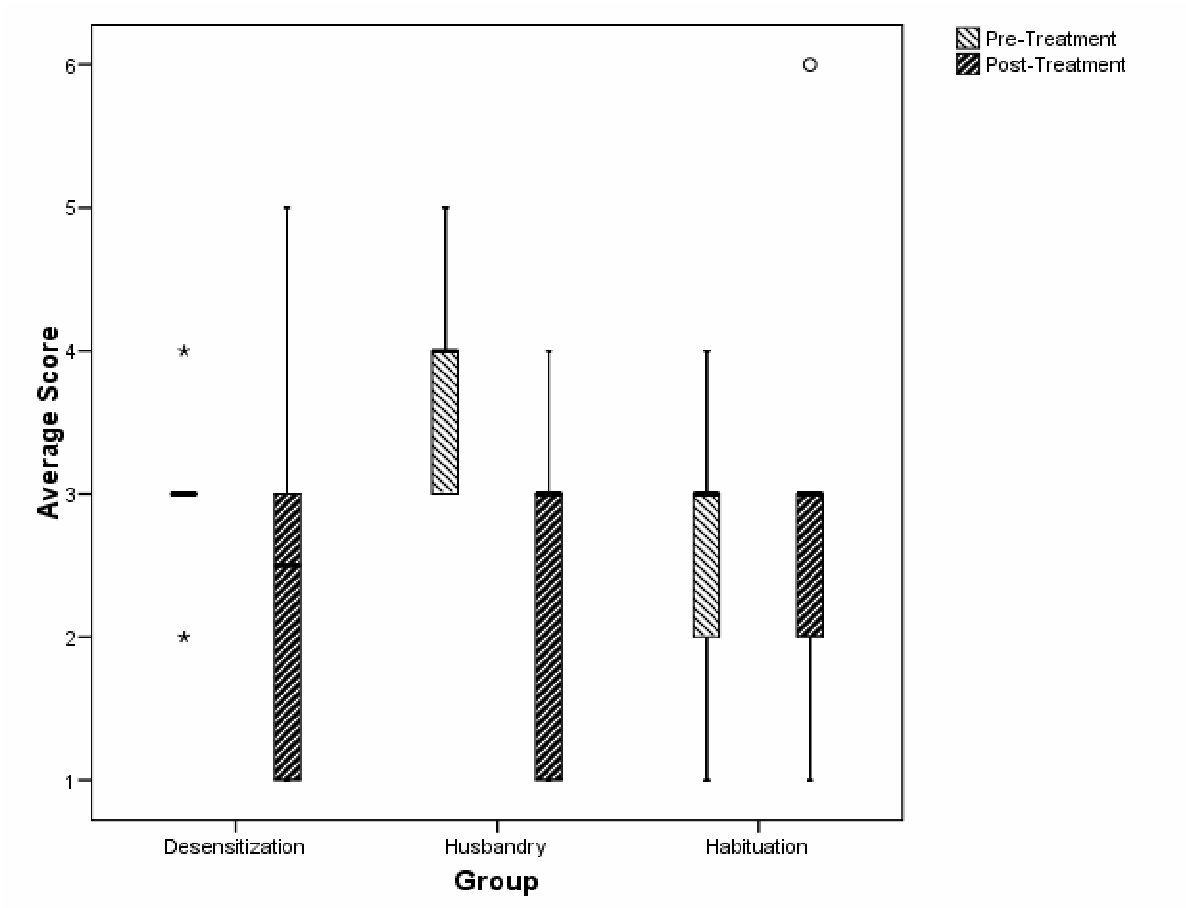


Figure Twenty-Three: Response Test Scores for Offering a Treat

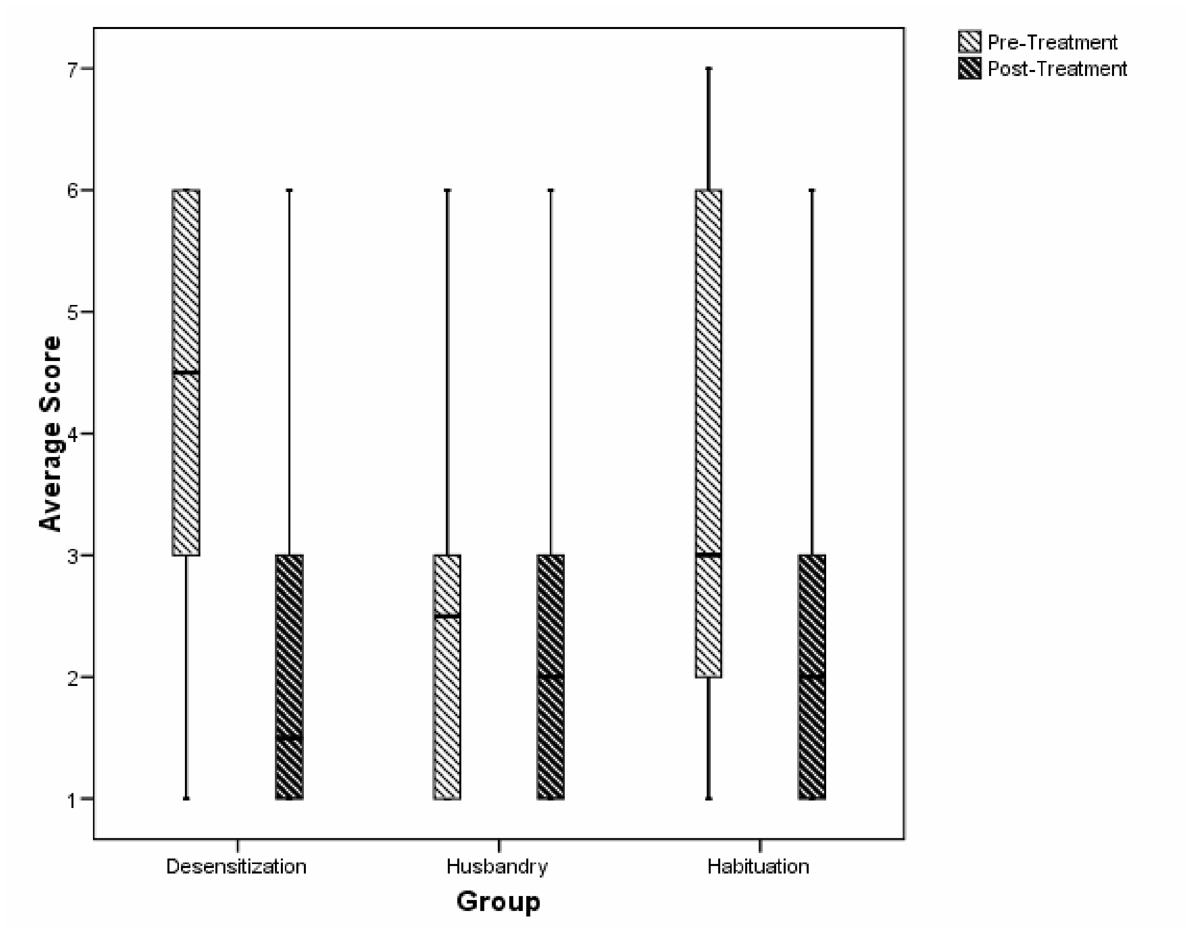
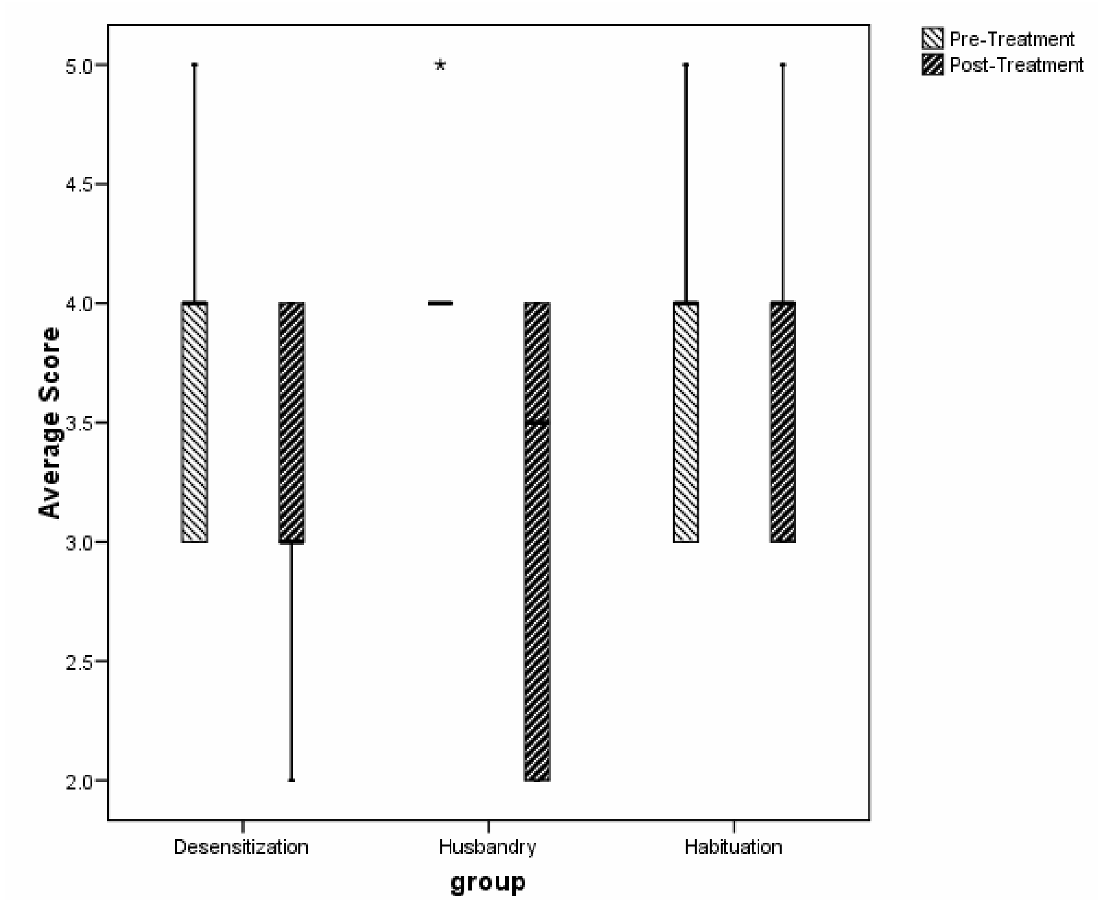


Figure Twenty-Four: Response Test Scores for Removal of Chow



CHAPTER 4

CONCLUSION

In summary, our findings indicate that desensitization reduced the rate of stress-related behaviors, cringing behaviors in general, and cringing behaviors as directed toward humans in a significant number of macaques for which this treatment was applied (for individual graphs, see Appendix F). Desensitization also reduced the duration of cringing as directed toward humans in a significant number of animals for which this treatment was applied. Neither husbandry training nor simple habituation to the environment reduced either the rate or duration of these behaviors in a significant number of subjects exposed to such treatment. The effect of treatment methods on the reduction of these behaviors was significant in terms of duration of time animals spent in the back of their cages and in terms of the duration of time the animals spent cringing toward humans. The results of this experiment, therefore, support the use of desensitization for reducing fear in singly-housed male rhesus macaques and indicate that this treatment is likely more effective than husbandry training or the simple exposure of the animal to the environment.

The reduction of stress following operant conditioning has been reported previously (Bassett, L., et al., 2003: McKinley, J., et al., 2003), but in this study we did not find stressful behaviors, cringing as directed towards humans, or cringing in general to be reduced for a significant number of animals in Group 2, which received basic husbandry training. This could be due to our small sample size. It could also be that desensitization does have a more robust effect on stress-related and fearful behaviors than basic husbandry training. Finally, some studies have indicated that the effects of positive human-animal interaction on stress-related physiological measures are variable (e.g., Nerem, R.M., Levesque, M.L., Cornhill, J.F., 1980). It could be that our data reflect behavioral variability in response to positive human-animal interaction, but further research is needed to clarify this relationship.

The control group in this experiment was assumed to experience habituation effects based merely on continued exposure to the environment over the course of the study. Habituation has been shown to be effective in the reduction of the fear-response, but not as effective as habituation combined with desensitization (Goldstein, A.J., et al., 1969; Webster, J., 1994). The desensitization group, in this experiment, necessarily was also undergoing habituation, not just to the environment, but also to human interaction and to the stimuli presented during training sessions. And, supporting Goldstein's (1969) results, the desensitization group did appear to experience a greater reduction in fearful and stress-related behavior than the habituation group over the course of the study.

Interestingly, changes in Response Test scores, which would logically have been larger for animals in the desensitization group, since they were being specifically desensitized to the stimuli that made up the test, were not notably different for those subjects as compared to husbandry subjects. This implies that something in the training methods other than the specific stimuli animals in Group 1 were desensitized to could actually have been more relevant to the reduction of fear-related behaviors. This could be further support for the impact of positive human interactions on behavior (e.g., Waitt, C., et al., 2002). Another strong possibility is that the effect of an unfamiliar person administering the second Response Test overrode any positive effects specifically attributable to the desensitization training. It may be advisable to include multiple trainers in future desensitization training procedures.

Finally, it could be that the animals in the desensitization group were being trained to engage in a certain behavior, and that this training had a greater impact on the animals' behavior than desensitization to the stimuli used in treatment. It is possible that the relevant factor in this case is the type of behavioral response that animals in Group 1 were given food rewards for, which was calm, non-fearful behavior. While animals in Group 2 were rewarded for certain behavioral responses, such as touching a target stick, there was little attempt to modify the nature of those responses. That is, if an animal fear-grimaced while touching the target, or touched the target in a somewhat threatening manner, no attempt was made to modify the attitude of the animal, unless the animal was extremely aggressive, in which case a short time out (5 seconds) was applied.

Considering the behavioral data and the Response Test data together, it seems reasonable to conclude that the fact that only animals in Group 1 were specifically rewarded for not reacting fearfully made a bigger difference in reducing those animals' fear-related behaviors than desensitizing those animals to the specific stimuli we had identified as probable fear triggers. Wolpe (1958, as cited by Callen & Boyd, 1990) advised that desensitization techniques should require, additionally, direct training of fear-antagonistic responses, such as eating (Wolpe, J., 1981) or relaxing. Perhaps, though inadvertently, the success in reducing fear-related behaviors for the desensitization group is attributable to training fear-antagonistic responses. This conclusion is supported by studies that have shown that desensitization procedures that employ a competing response are more effective than extinction procedures, which do not (Sue, D., 1975).

The correlation between loud noise and stressful behavior, but not between human activity triggers and either fearful or stressful behaviors, indicates that stress is more heavily impacted by noise (and perhaps noisy human activity, as well as noisy conspecific or machinery-based activity) than it is by humans being in the area or working in the area, as long as whatever they are doing is not loud. We did find that the desensitization group experienced a significant reduction in the rate of stress-related behaviors, while the husbandry and control groups did not, but there was no attempt in this study to control for loud noise, so we cannot be sure that noise was not a significant factor in any observed changes in stress-related behaviors.

The treatment-associated changes in the amount of time that animals spent in the backs of their cages are interesting. Usually, when fearful animals are cringing, they are doing so in an uppermost back corner of their cage. Therefore, the finding that animals in both training groups reduced the amount of time spent in the backs of their cages (and thus necessarily increased the amount of time spent in the fronts of their cages), while control group animals actually increased the amount of time spent in the backs of their cages indicates that the training group animals were perhaps spending less time cringing, or were generally less afraid of their environment. It is also possible that training was responsible for this change for a more parsimonious reason – the animals in training groups were being rewarded when sitting in the front of their cages during training sessions, and this response generalized to time outside of training sessions.

Temperament Test results did not turn out to be supportive of our initial hypothesis in terms of effects on fear-related behaviors. However, this could have been due to covariance issues, confounds due to group assignment, or any number of factors. To better test the effects of temperament on the effectiveness of behavior modification, it would be advisable to match pairs across treatment groups based on temperament ratings, assuring that each treatment group had an equal number of subjects representing each temperament category. We did not do that for this experiment. However, we were able to confirm previous studies' claims to the extent that animals of relatively more inhibited temperaments also seemed to learn at a slower rate (Coleman, K., et al., 2005).

Data in support of our primary hypothesis, that desensitization would be more effective in reducing fear-related behaviors than either husbandry training or simple habituation, seem to be reasonably strong. However, to further explore this issue, larger sample sizes would likely both increase the power of statistical tests and decrease covariance problems. It is recommended that any future research also make sure to take into account the effects of loud noise on stress-related behaviors and either plan to account for that with regression or analysis of covariance techniques or control for it in some other fashion. It could be beneficial to add a treatment group in which animals are rewarded merely for calm, non-fearful behavior, with no specific stimuli introduced for desensitization purposes. It would also be interesting to have more than one trainer for each animal in some treatment groups to see if that enhances generalization of fear-reduction.

CHAPTER 5

DISCUSSION

Animal welfare has been described as meaning something that "includes the animals not being fearful (Dawkins, M.S., 2004)." This sentiment is reflected in the inclusion of "freedom from fear and distress" in the Five Freedoms (Farm Animal Welfare Council, 1993, as cited by Webster, 2005) (see Appendix E for a complete list). We do not currently have clear evidence to indicate how much of captive animals' fear is related to human activity, but some have suggested that the animals' responses' to humans could be, in itself, an adequate measure of fear (Jones, 1997). Hediger (1950) suggested that fear of humans could be one trait that predisposes some species to poor captive welfare. If desensitization procedures can effectively reduce the fear a captive animal expresses towards its human caregivers, this could arguably be a worthwhile method for improving captive animal welfare. A prototype for welfare assessment developed for dairy and pig farms includes special tests measuring animals' fearfulness of humans, administered four times a year (Sorenson, J.T., Sandoe, P., & Halberg, N., 1998, as cited by Johnsen, P.F., Johannesson, T., & Sandoe, P., 2001). Similarly, fear inventories have been used to assess anxiety in human patients (e.g., Wolpe, J., & Lang, P.J., 1964). Perhaps equivalent tests could be applied by behavioral management programs in laboratories and zoos.

It seems advisable to work toward individualizing this kind of assessment and treatment. This is critical to determining methods for successfully reducing fearful behavior (Wolpe, J., 1986). For some subjects in this study, we may have failed to properly identify the stimuli that elicited fearful behavior, or may not have individualized treatment to the capacity that the animals required. As Wolpe (1986) states: "To plan the extinction even of a simple conditioned salivary response requires the identification of the antecedent stimuli in order to ensure their presence in the extinction procedure; and this applies equally to the elimination of every unadaptive habit, no matter how complex."

Stress is also related to animal welfare, and though many of the variables relating to stress and the effects of stress on physiology remain unclear, the negative impact of high levels of stress has been demonstrated repeatedly, including (but not limited to) impaired learning ability, impaired memory, and damage to the hippocampus (for a review, see Sapolsky, R., 1992). It seems that desensitization training may effectively reduce stress for singly-housed rhesus, but it would be valuable to further delineate the effects of loud noise on stress, perhaps including some physiological measures to verify what we have seen behaviorally.

Finally, because the desensitization and husbandry training procedures used for this experiment were of relatively low cost, both in terms of time and equipment, applying similar techniques, particularly for animals that are transitioning from group-housing to single-housing, could be of benefit to the animals and to the researchers who plan to work with the animals. Each animal in the desensitization group received a total of barely more than two hours of training time, and the effects of this training seem to be positive. Future research will hopefully explore this issue further, but until then, implementing some similar procedure might be of use for laboratories that have singly-housed rhesus macaques.

APPENDIX A

TEMPERAMENT ASSESSMENT

Initial Response to Observer:

Ignore you (1)   Normal Behaviors (2)   Aggressive, threat (3)   Avert Eyes (4)   Fear (5)

Predominant Response during Observation (5 Minutes):

Ignore you (1)   Normal Behaviors (2)   Aggressive, threat (3)   Avert Eyes (4)   Fear (5)

Initial Response to Direct Eye Contact

Ignore you (1)   Normal Behaviors (2)   Aggressive, threat (3)   Avert Eyes (4)   Fear (5)

Predominant Response to Direct Eye Contact (2 minutes):

Ignore you (1)   Normal Behaviors (2)   Aggressive, threat (3)   Avert Eyes (4)   Fear (5)

Circle Initial Response to Novel Food (5 minutes, time each response latency in seconds):

Take food (1)   Inspect food (2)   Threat, Aggressive (3)   Avert Eyes (4)   Fear (5)

Latency to: Inspect ____________ Touch _____________ Manipulate ___________

Circle Initial Response to Novel Object (5 minutes, time each response latency in seconds):

Grab object (1)   Inspect object (2)   Threat, Aggressive (3)   Avert Eyes (4)   Fear (5)

Latency to: Inspect ____________ Touch _____________ Manipulate ___________

APPENDIX B

RESPONSE TEST

Rating System:

- 1 = no response, animal remains still, appears unconcerned; for treat offering, takes treat immediately, calmly, without threatening
- 2 = moves away from stimulus, otherwise unconcerned; for treat offering, takes treat calmly but not right away
- 3 = moves away from stimulus, piloerect, may open mouth stare; for treat offering, threatens before or after taking food, may be piloerect
- 4 = moves away from stimulus, piloerect, open mouth stare, grabs at stimulus or otherwise threatens; for treat offering, threatens and snatches food in an aggressive manner
- 5 = moves away from stimulus, freezes, cringes, and/or averts eye gaze; for treat offering, very slow to take treat, takes treat from back corner of cage, possibly averts eye gaze
- 6 = freezes and/or cringes and fear grimaces in response to stimulus; does not take treat
- 7 = freezes and/or cringes, fear grimaces, and scream/shrieks in response to stimulus; does not take treat

Test Stimuli:

- Insertion of chow stick into cage to check Lixit ________________
- Offer of treat at front of cage (30 sec only) ____________________
- Spraying of floor in front of cage with hose ___________________

- Removal of excess chow using chow stick ______________________

APPENDIX C

ETHOGRAM FOR BEHAVIORAL OBSERVATIONS

Continuous Sampling: record priority behavior on each channel

<u>Location Channel:</u> Front Half versus Back Half of Cage

<u>Behavioral Channel:</u>

Abnormal Behaviors

- Bizarre posture: holding seemingly uncomfortable or unnatural position
- Coprophagy: ingesting feces
- Eye poke: digit in eye socket
- Feces paint: smearing feces on a surface
- Flip: flipping body vertically in circles in cage, not recorded if part of a pacing routine that includes pacing around cage horizontally
- Floating limb: limb raised in air, appearing as if not in control of it
- Head toss: movement of head like a short tic in a repetitive manner, not recorded if part of a pacing routine
- Masturbate: manipulation and/or fondling of genitals, including self-fellatio
- Overgroom: plucking of hair from body
- Pace: repetitive, stereotyped locomotion, primarily horizontal, must repeat the same locomotory path 2x before it can be recorded as pacing and not locomotion
- Deposit food or water and reingest: depositing food (not vomit) or water on substrate with the mouth and reingesting
- Regurgitate and reingest: same as above but with vomit

- Rock: swaying of upper body back and forth in a repetitive manner, animal can be sitting or standing; not recorded if part of a pacing pattern
- Self-bite: biting of one's own body in an aggressive manner, may or may not cause injury
- Self-clasp: clutching of one's own body with hand(s) and/or foot(feet)
- Self-mouth: involves placement of a part of subject's body into its mouth (sucking of fingers, toes, or scrotum)
- Self-slap: slapping of one's own body in a manner that would seem to be harmful or painful
- Stereotypy: any repetitive behavior with no obvious function that is not specified
- Urine drink: licking or sucking of pooled urine from a surface or directly from penis

Enrichment-Directed Behavior:

- Attack enrichment: bite or grab enrichment in an aggressive manner
- Display with enrichment: use enrichment to threaten by shaking, throwing, etc.
- Manual/oral enrichment manipulation: touch, handle, forage, chew, bite, lick, suck enrichment

Other nonsocial behaviors:

- Eat/drink: does not include manipulating food object in mouth – recorded as object manipulation unless actively eating
- Inactive and passive visually: sleeping or just sitting and not actively looking at anything
- Locomotion: walk, climb, jump, 2 steps or more

- Manipulate other: using hands, feet or mouth to explore inanimate objects other than enrichment, includes manipulation of food and holding food
- Huddle: sustained inactivity in contact with object or caging
- Visual scanning: sits or stands or looks out into the room in deliberate manner and without aggression

Self-Directed behavior:

- Body shake (considered a stress-related behavior in analysis): rapid shaking of head and shoulders
- Bite nails: chewing of nails non-aggressively
- Lick self: sniffing or touching tongue to part of body
- Manipulate self: exploring, pushing, pulling or moving some small part of the body (not genitals)
- Scratch (considered a stress-related behavior in analysis): vigorous strokes of hair, more than one motion
- Self-groom: any picking, stroking, and/or licking of one's own body hair (more than one motion, plucking hair = overgroom)
- Self-play: any repetitive activity that involves a toy or part of the cage, active and vigorous

Social Behaviors (modifier om = other monkey, modifier pr = human in room, modifier uk = unknown):

Affiliative:

- Affiliative contact: grooming, grooming motions, non-aggressive physical contact
- Attempt to touch: try to touch recipient non-aggressively

- Lip-smack: bringing lips together rapidly, resulting in smacking sound; teeth are covered
- Present: present belly, neck, or other part of body for grooming
- Coo calls (vocal behavior): a clear tone of medium pitch and intensity; mouth open in a diamond shape
- Grunts (vocal behavior): short, repetitive, low guttural sounds

Aggressive:

- Bite: bite or attempt to bite another animal or human
- Bob: a rapid up and down motion of upper part of the body on flexed limbs, sometimes involves only the head
- Cage shake: any vigorous shaking of cage
- Crook tail: strutting type of locomotion with tail held high and curled at the end, can also be seen when animal is stationary
- Ear flick and/or raised eyebrows: quick flattening of ears against scalp and/or scalp retracted to expose eyelids; if accompanied by open mouth stare, record stare
- Grab at: aggressive touch or attempted aggressive touch of another animal or human
- Jaw snap: sound made when bringing lower and upper jaws together quickly and repetitively
- Lunge: high-speed aggressive intention movement toward another animal or human

- Open mouth stare: visual fixation in an aggressive context; animal's head thrust forward and body appears rigid
- Teeth grinding: grinding of upper and lower jaws together
- Yawn: (considered a stress-related behavior for purposes of analysis)
- Bark (vocal behavior): hoarse, staccato low sounds

Submissive (fear-related behaviors):

- Fear grimace: a grin-like facial expression involving retraction of the lips exposing clenched teeth; may be accompanied by flattened ears, stiff-huddled body posture, and screeching vocalizations
- Rapid glances: nervous, repetitive, rapid pivoting of head in an attempt to look at another animal or human without risking eye contact
- Cringe: a posture involving a crouched position where limbs are held beneath the body and head is lowered; also includes animal pressing body into back corner of the cage, usually a top corner.
- Rump present: a posture involving a stance on all fours with the hind quarters elevated and the tail raised or to the side; may be accompanied by brief tail flicks
- Freeze: animal remains motionless for 5 seconds or more, frozen in position rather than merely idle (record only if not in conjunction with cringing)
- Scream/shriek (vocal behavior): shrill, high-pitched, high intensity, multi-toned sounds

Other: any behavior not listed on the ethogram

Unclear: cannot see what animal is doing

Priority rules:

Abnormal>social>enrichment-directed>vocal>self-directed>other nonsocial

Fear Trigger Channel:

Record the following events as they occur (10 second rule: if event recurs within 10 seconds, do not record as second instance of event):

- Approach: human approaches within 3 feet of cage front
- Add food or enrichment: human puts food or enrichment item into or onto cage
- Insertion of chow stick to cage: human approaches and inserts chow stick into cage either to check Lixit or to remove excess chow
- Loud Noise: any loud, abrupt noise in the room, can result from animals banging cages or from door slamming

APPENDIX D

TRAINING PROCEDURES

Desensitization Training Procedures

A. Clicker Training Steps

1) click, then offer treat

2) animal does not threaten or indicate fear when hears clicker

3) animal indicates expectation of treat when clicked

B. Lixit Checking Training Steps

1) hold stick at side; animal to sit near front of cage and accept treats

2) move stick up in degrees, same response to be rewarded

3) hold stick at front of cage

4) slide stick into cage in degrees

5) slide stick into cage and touch Lixit, activate Lixit

6) slide stick into cage less slowly, in degrees, until able to complete action at a regular speed

7) complete whole process with more banging and carelessness associated

C. Chow Removal Training Steps

1) hold stick at side; animal to sit near front of cage and accept treats

2) move stick up in degrees, same response to be rewarded

3) hold stick (flat end closest) at front of cage

4) slide into cage in degrees, flat end first

5) remove chow from cage slowly, in degrees, until able to complete action at regular speed

6) move toward doing process more quickly and with less care

D. Spray Hose Training Steps

1) hold hose at side in front of cage

2) spray floor briefly, then extend time spraying

3) move hose around, spraying more of the floor

4) spray in front of cage

E. Treat Offer Training Steps

1) hold treat in hand at front of cage, wait for animal to sit calmly (not threaten, flinch or grab), then offer treat

2) in degrees, work toward moving hand more quickly to front of cage

Husbandry Training Procedures

A. Clicker Training Steps

1) click, then offer treat

2) animal does not threaten or indicate fear when hears clicker

3) animal indicates expectation of treat when clicked

B. Target Training Steps

1) reward animal for looking at target

2) reward animal for touching target

3) reward animal for moving to touch target at a new location

C. Stationing Training Steps

1) reward animal for coming to front of cage

2) reward animal for sitting at front of cage

3) reward animal for hold target

4) reward animal for holding target for longer times

D. Present Rear Training Steps

1) reward animal for approximation toward presenting rear

2) reward animal for allowing touch to rear

3) reward animal for allowing longer touch to rear while remaining motionless

E. Present Shoulder Training Steps

1) reward animal for sitting with shoulder at mesh

2) reward animal for putting shoulder closer and closer to mesh

3) reward animal for allowing touch to shoulder

4) reward animal for allowing longer touch to shoulder while remaining motionless

APPENDIX E

THE FIVE FREEDOMS

1) Freedom from thirst, hunger, and malnutrition

2) Freedom from discomfort

3) Freedom from pain, injury and disease

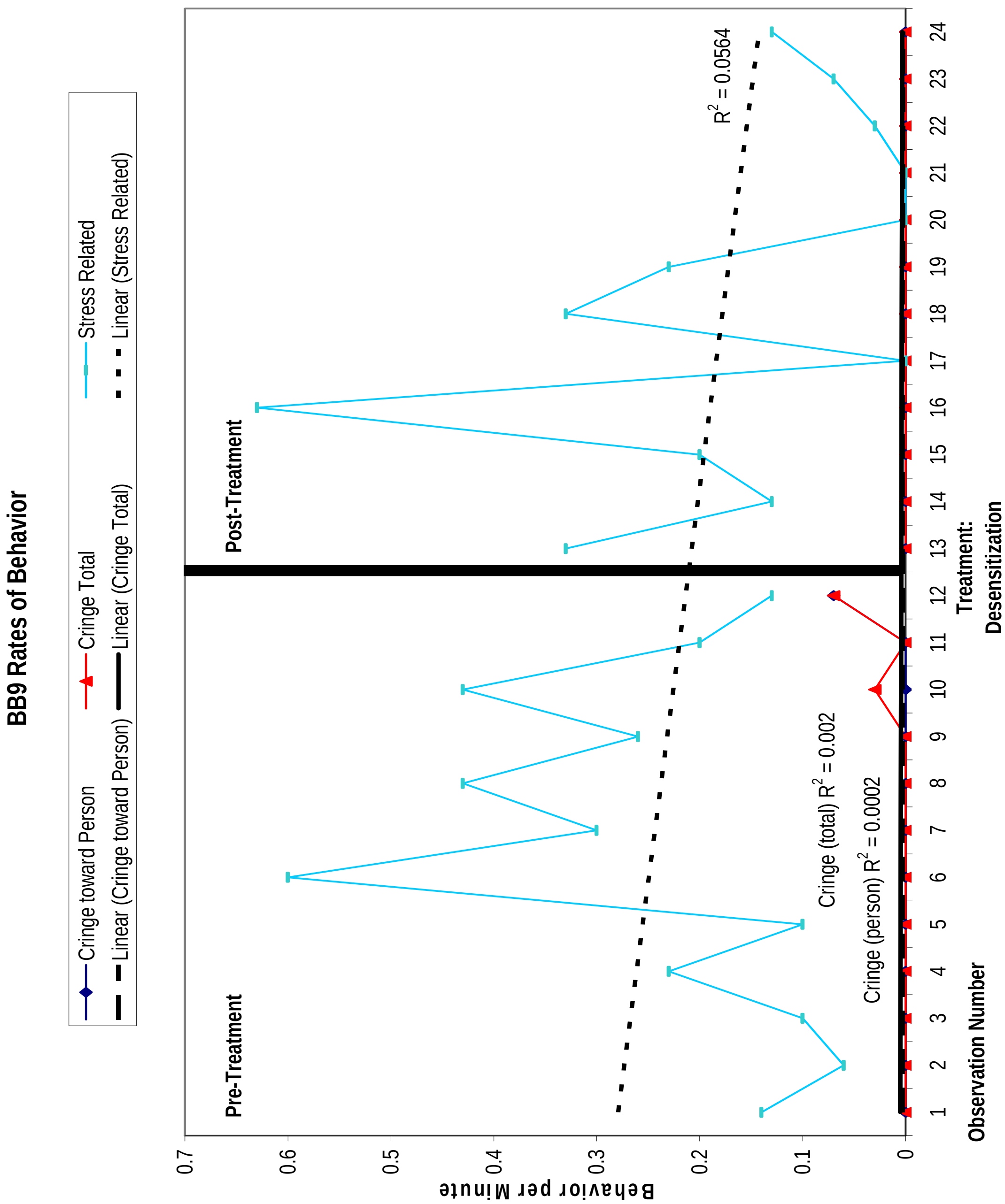
4) Freedom from fear and distress

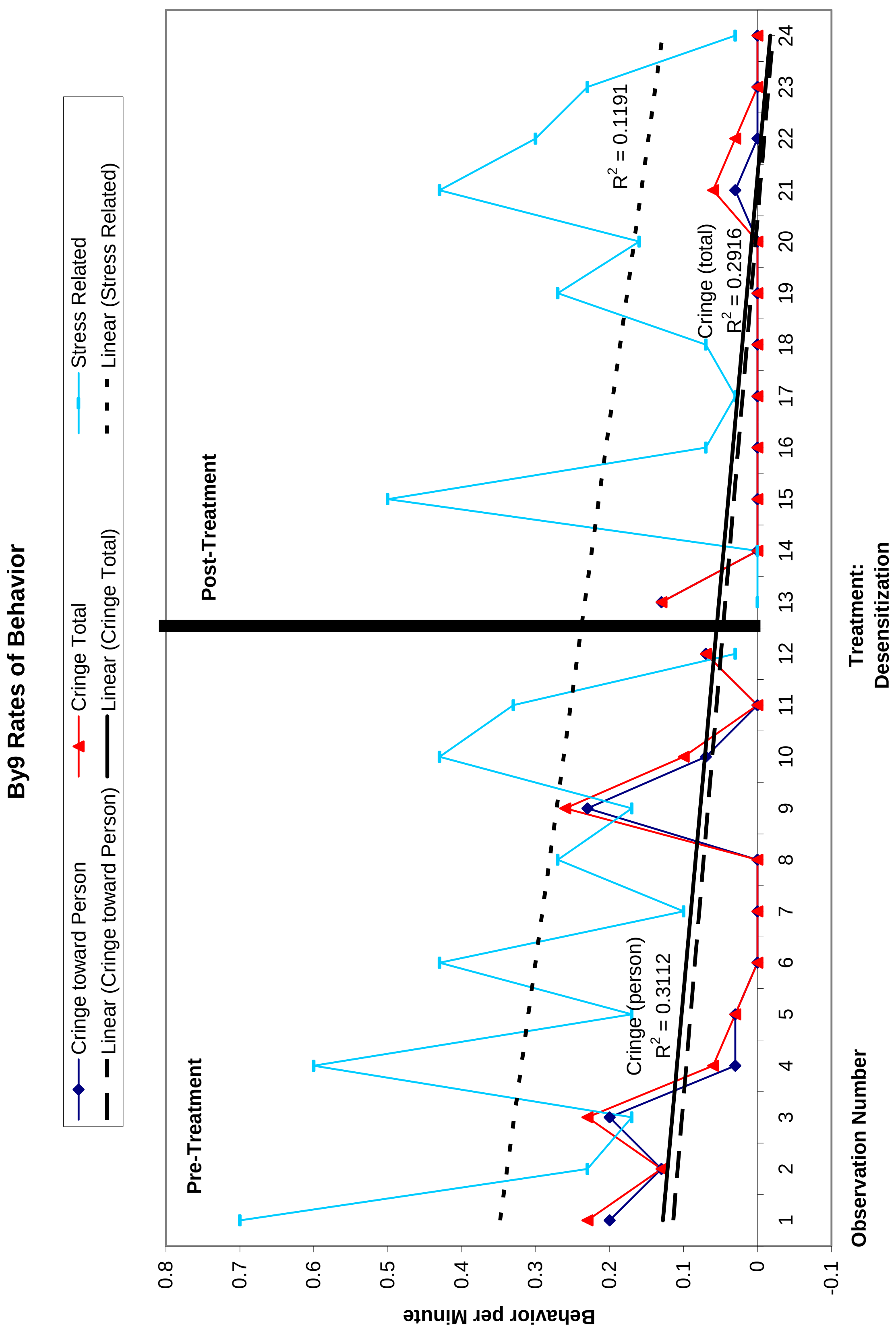
5) Freedom to express normal behavior

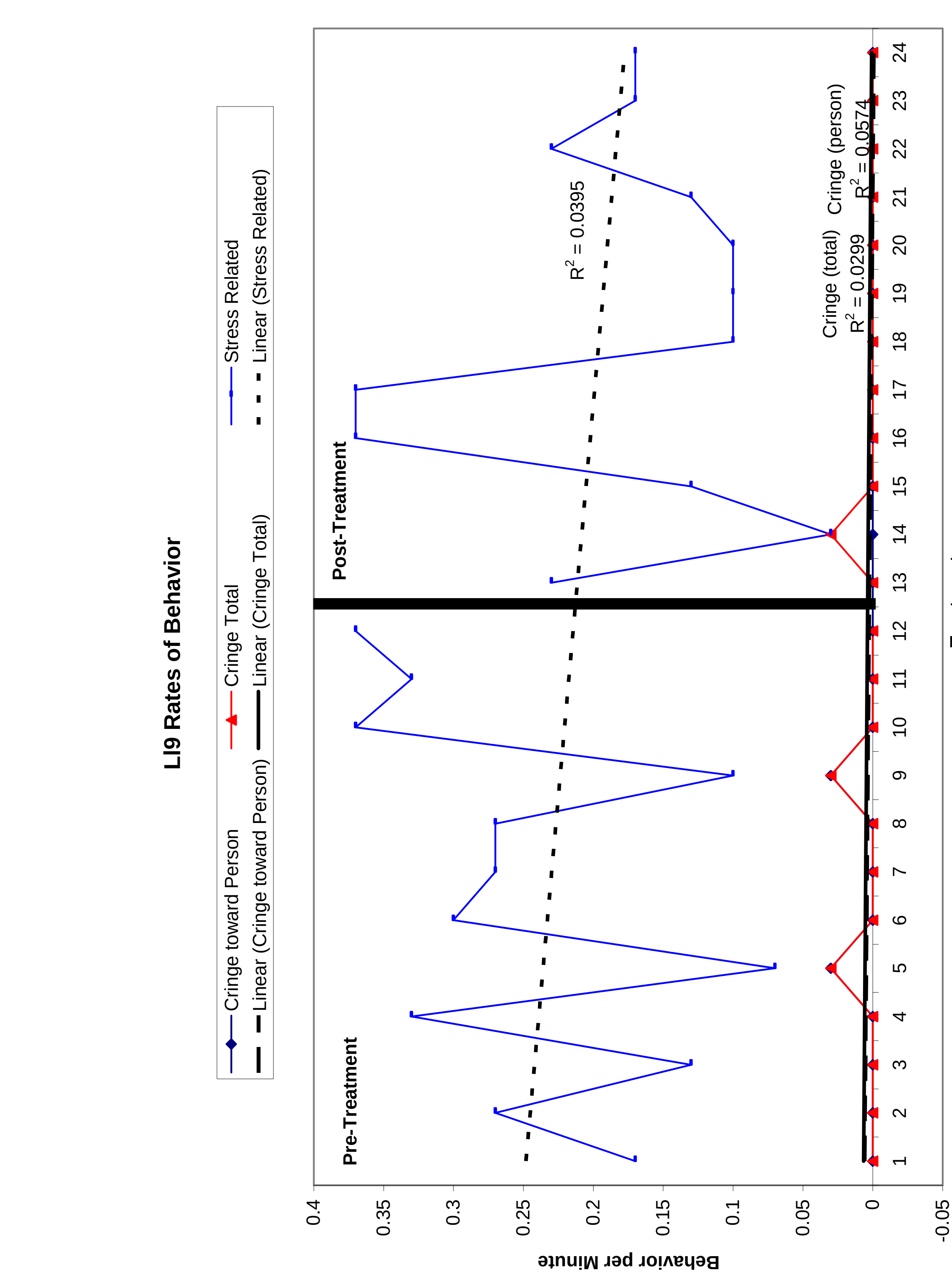
APPENDIX F

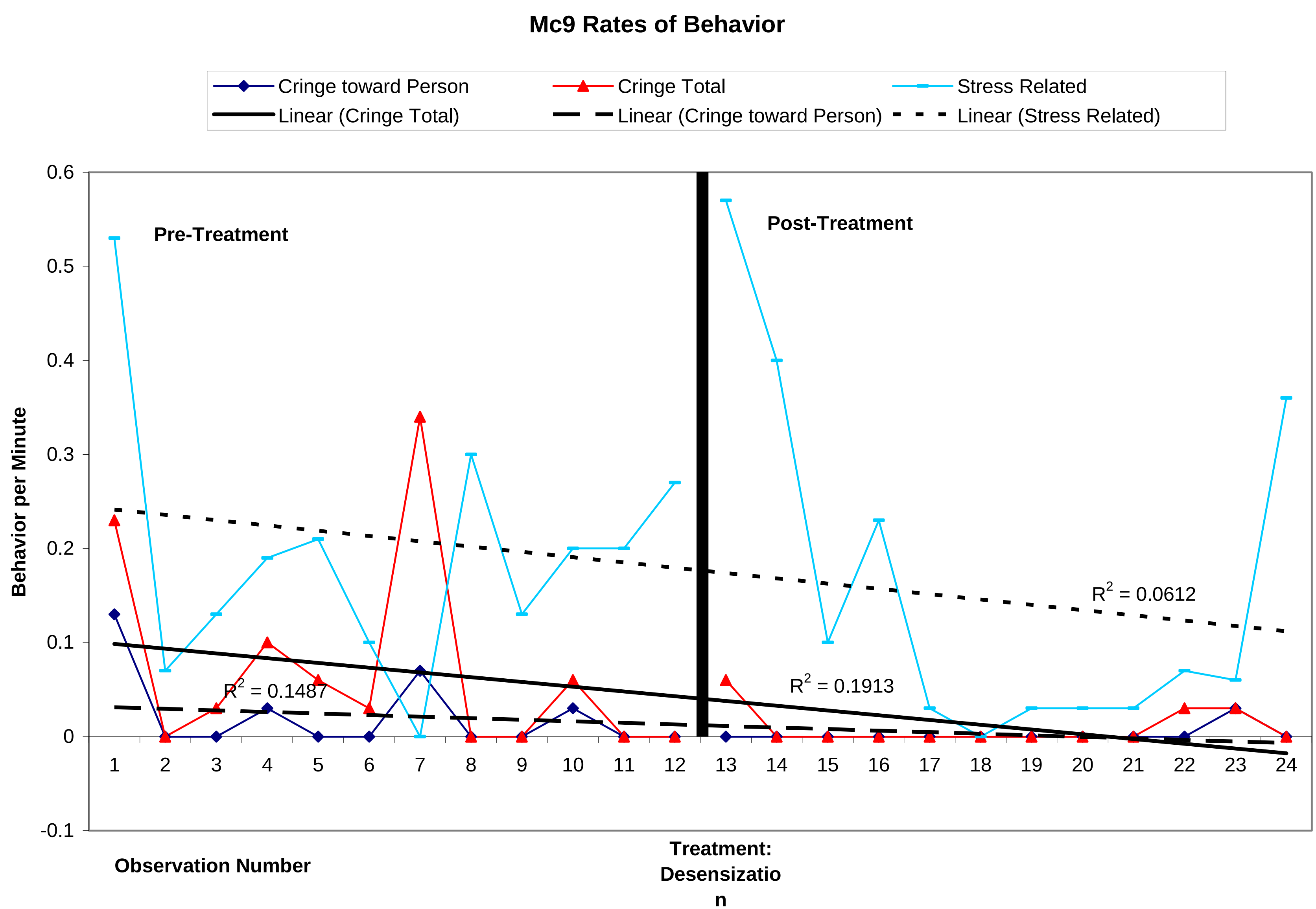
INDIVIDUAL BEHAVIOR GRAPHS

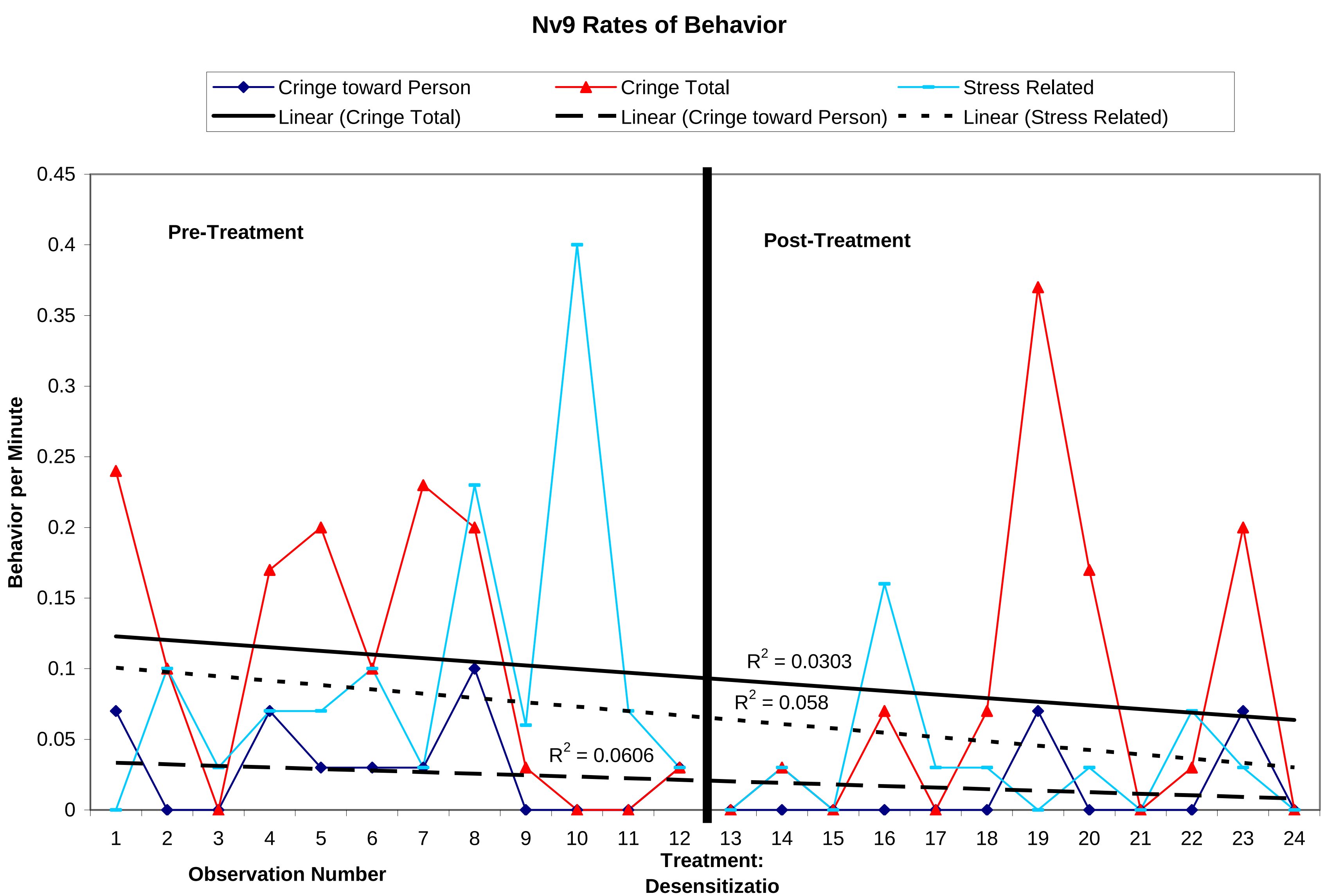
Desensitization Group: Rates of Behavior

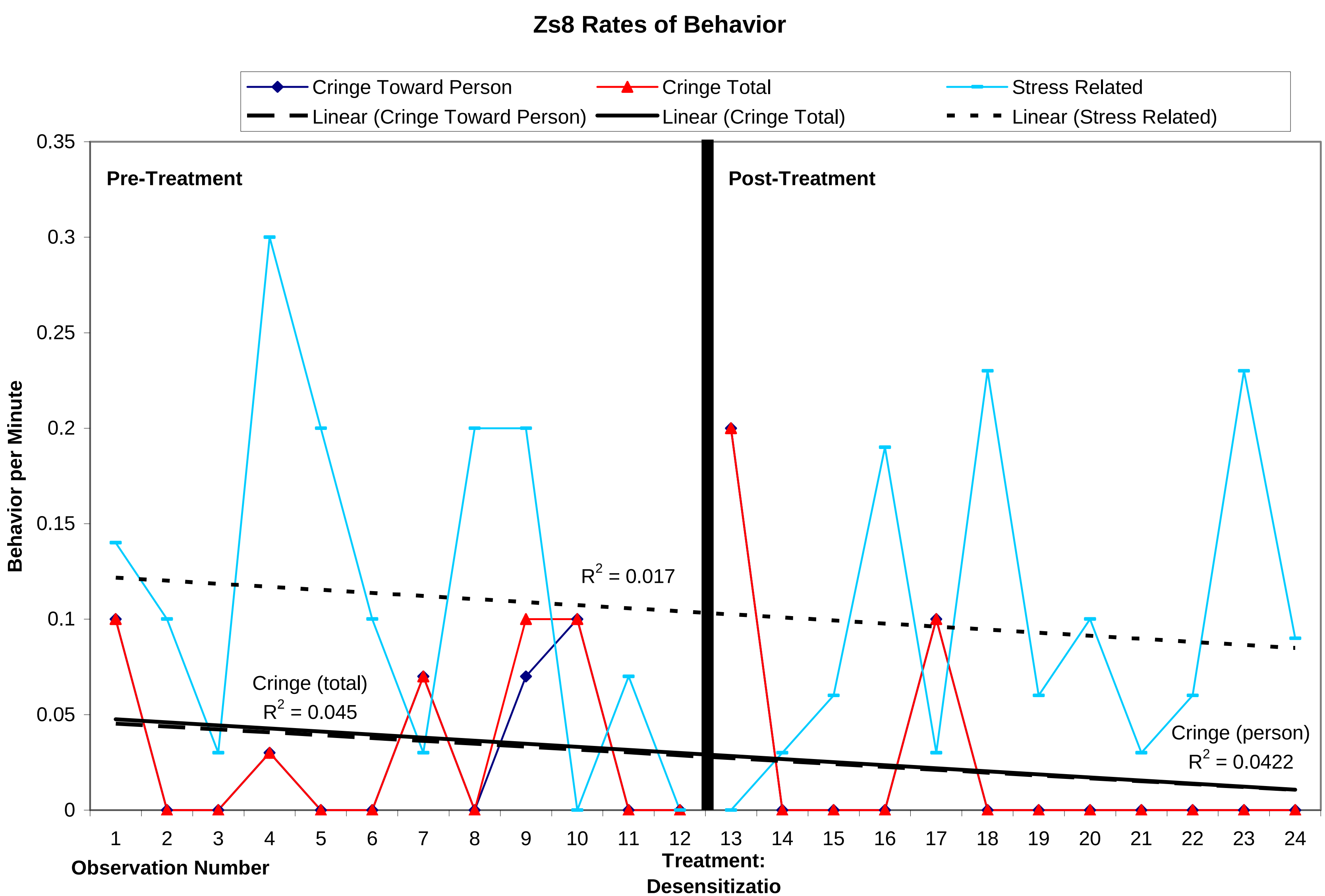



By9 Rates of Behavior
Cringe toward Person
Cringe Total
Stress Related
Linear (Cringe toward Person)
Linear (Cringe Total)
Linear (Stress Related)
Pre-Treatment
Post-Treatment
Cringe (person)
R2 = 0.3112
Cringe (total)
R2 = 0.2916
R2 = 0.1191
Behavior per Minute
Observation Number
Treatment:
Desensitization
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
-0.1
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24

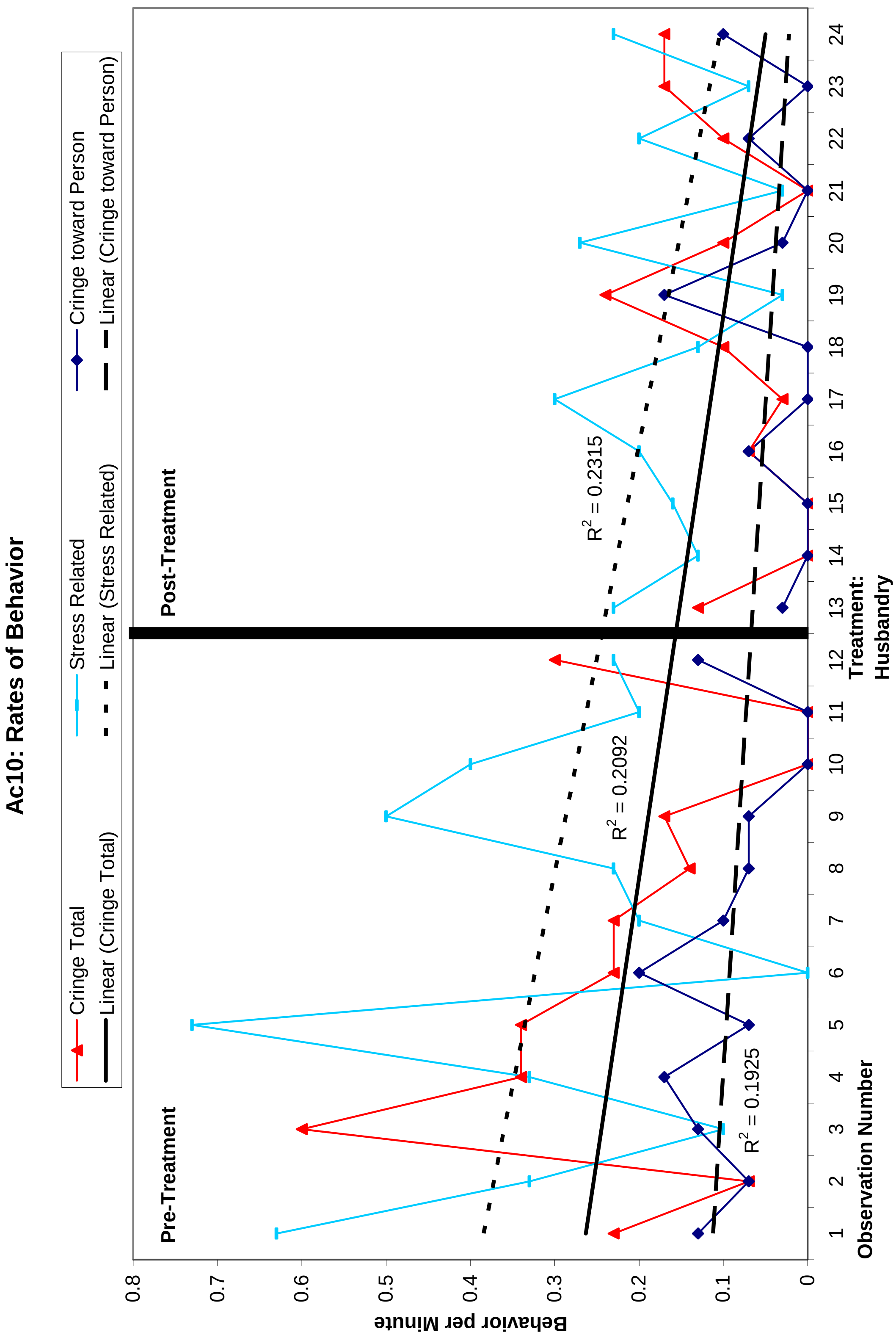

LI9 Rates of Behavior
Cringe toward Person
Cringe Total
Stress Related
Linear (Cringe toward Person)
Linear (Cringe Total)
Linear (Stress Related)
Pre-Treatment
Post-Treatment
$R^2 = 0.0395$
Cringe (total)
$R^2 = 0.0299$
Cringe (person)
$R^2 = 0.0574$
Behavior per Minute
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
-0.05
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
Observation Number
Treatment:
Desensitization

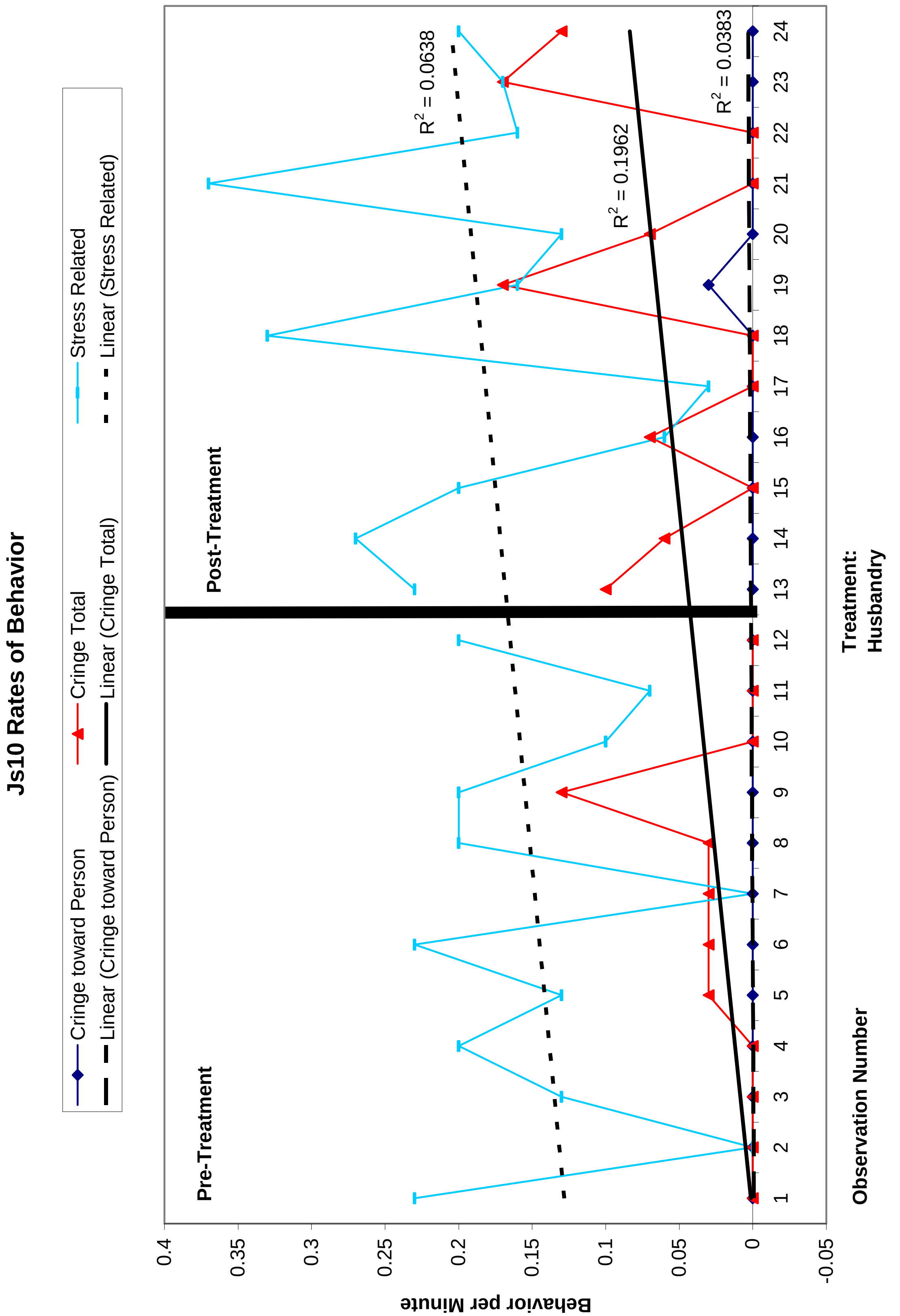

Mc9 Rates of Behavior
Cringe toward Person
Cringe Total
Stress Related
Linear (Cringe Total)
Linear (Cringe toward Person)
Linear (Stress Related)
Behavior per Minute
0.6
0.5
0.4
0.3
0.2
0.1
0
-0.1
Pre-Treatment
Post-Treatment
R2 = 0.1487
R2 = 0.1913
R2 = 0.0612
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
Observation Number
Treatment: Desensizatio n

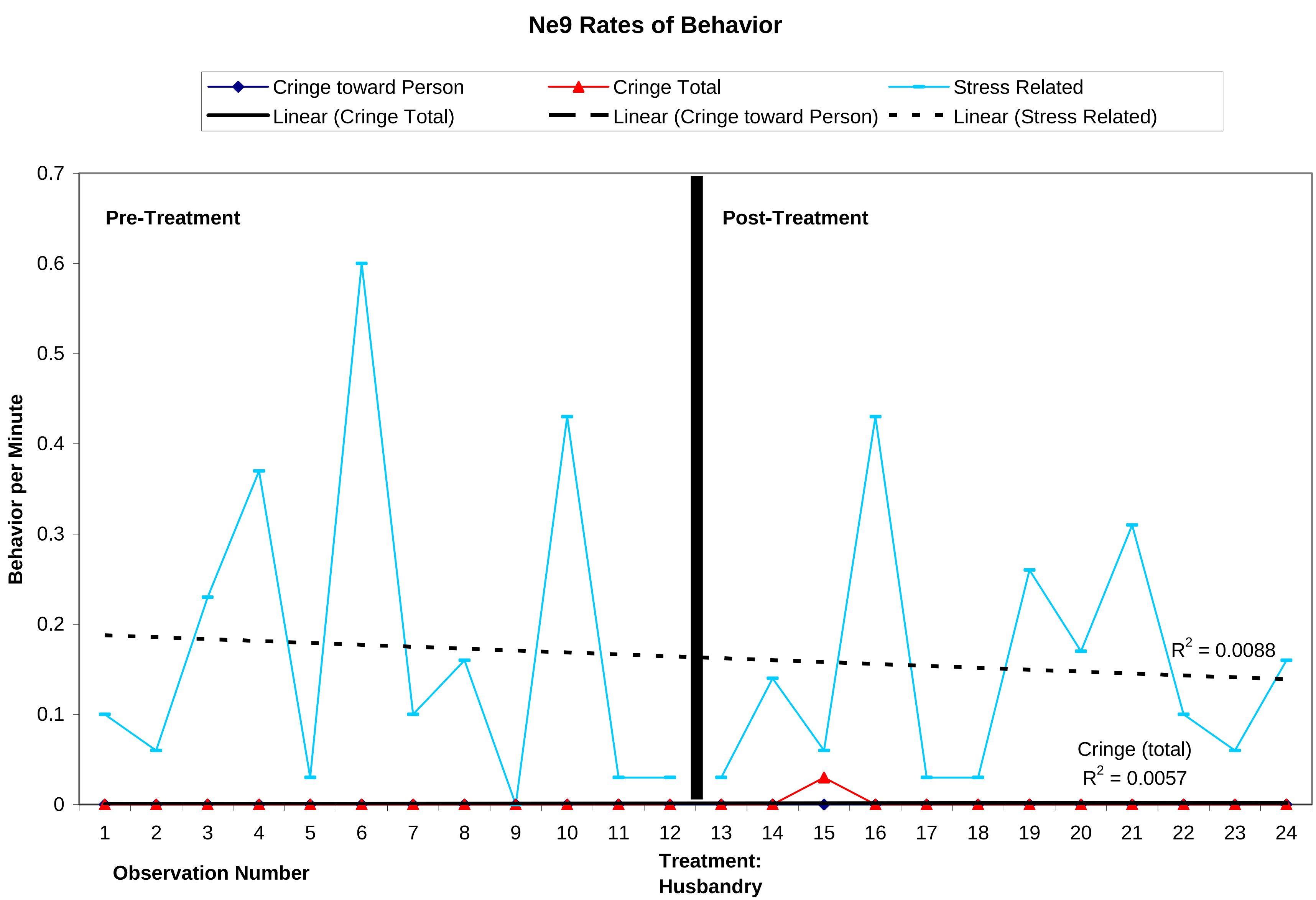

Nv9 Rates of Behavior
Cringe toward Person
Cringe Total
Stress Related
Linear (Cringe Total)
Linear (Cringe toward Person)
Linear (Stress Related)
Behavior per Minute
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
Pre-Treatment
Post-Treatment
$R^2 = 0.0303$
$R^2 = 0.058$
$R^2 = 0.0606$
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
Observation Number
Treatment:
Desensitizatio


Zs8 Rates of Behavior
Cringe Toward Person
Cringe Total
Stress Related
Linear (Cringe Toward Person)
Linear (Cringe Total)
Linear (Stress Related)
Behavior per Minute
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
Pre-Treatment
Post-Treatment
R2 = 0.017
Cringe (total)
R2 = 0.045
Cringe (person)
R2 = 0.0422
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
Observation Number
Treatment:
Desensitizatio

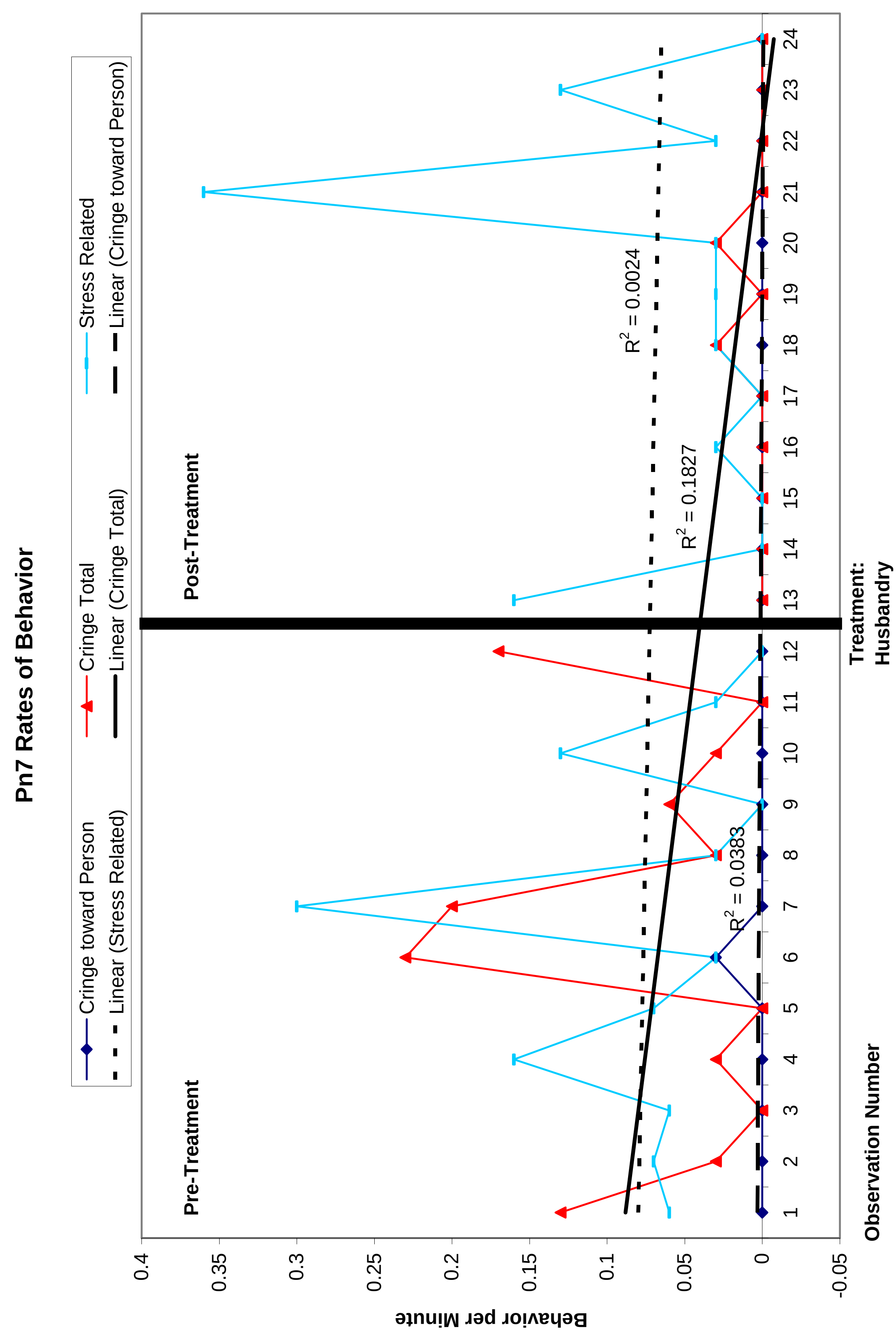
Husbandry Group: Rates of Behavior

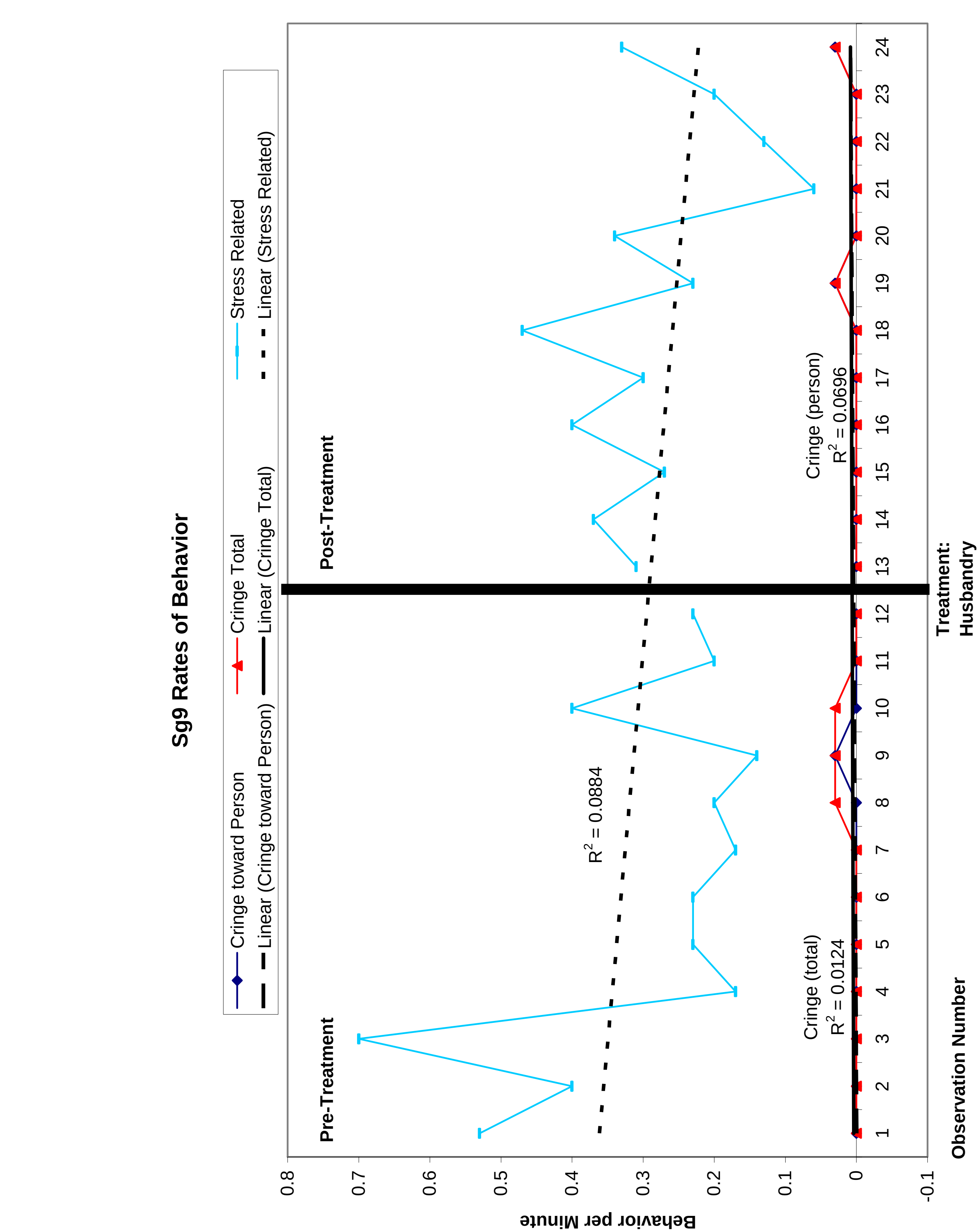


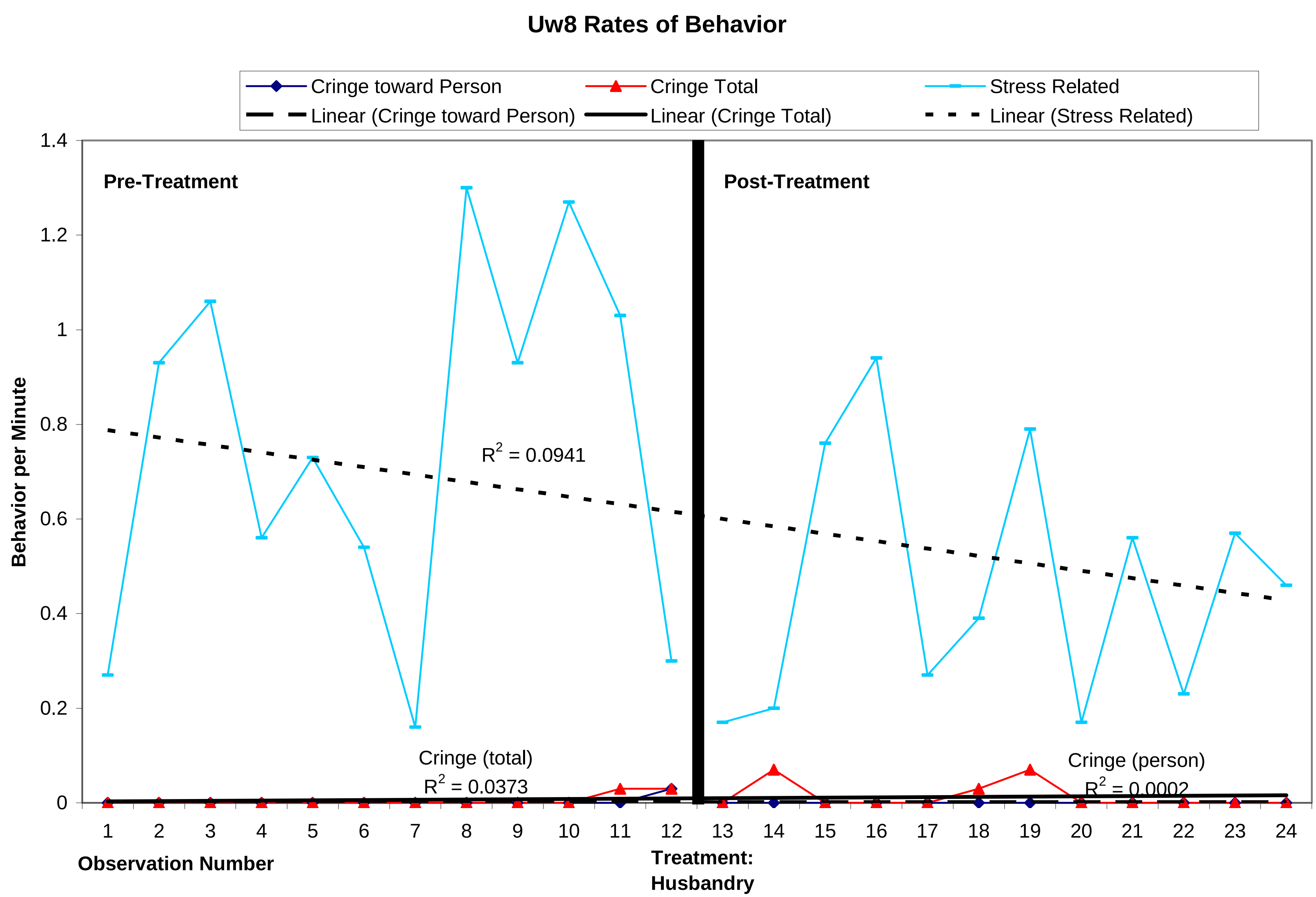

Js10 Rates of Behavior
Cringe toward Person
Cringe Total
Stress Related
Linear (Cringe toward Person)
Linear (Cringe Total)
Linear (Stress Related)
Pre-Treatment
Post-Treatment
$R^2 = 0.0638$
$R^2 = 0.1962$
$R^2 = 0.0383$
Behavior per Minute
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
-0.05
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
Observation Number
Treatment: Husbandry



Ne9 Rates of Behavior
Cringe toward Person
Cringe Total
Stress Related
Linear (Cringe Total)
Linear (Cringe toward Person)
Linear (Stress Related)
Behavior per Minute
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Pre-Treatment
Post-Treatment
R^2 = 0.0088
Cringe (total)
R^2 = 0.0057
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
Observation Number
Treatment:
Husbandry

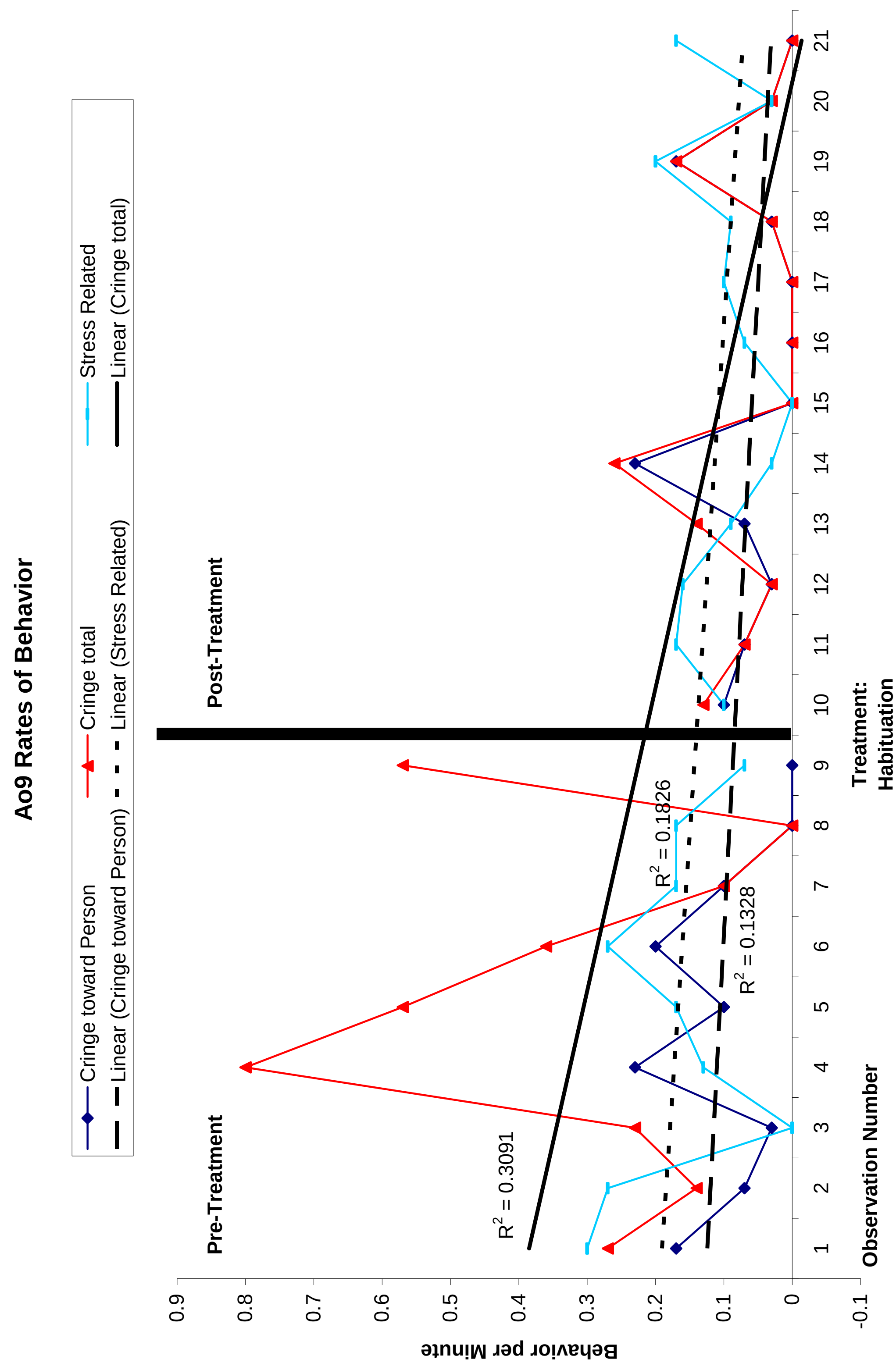

Pn7 Rates of Behavior
Cringe toward Person
Cringe Total
Stress Related
Linear (Stress Related)
Linear (Cringe Total)
Linear (Cringe toward Person)
Behavior per Minute
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
-0.05
Pre-Treatment
Post-Treatment
R^2 = 0.0383
R^2 = 0.1827
R^2 = 0.0024
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
Observation Number
Treatment:
Husbandry

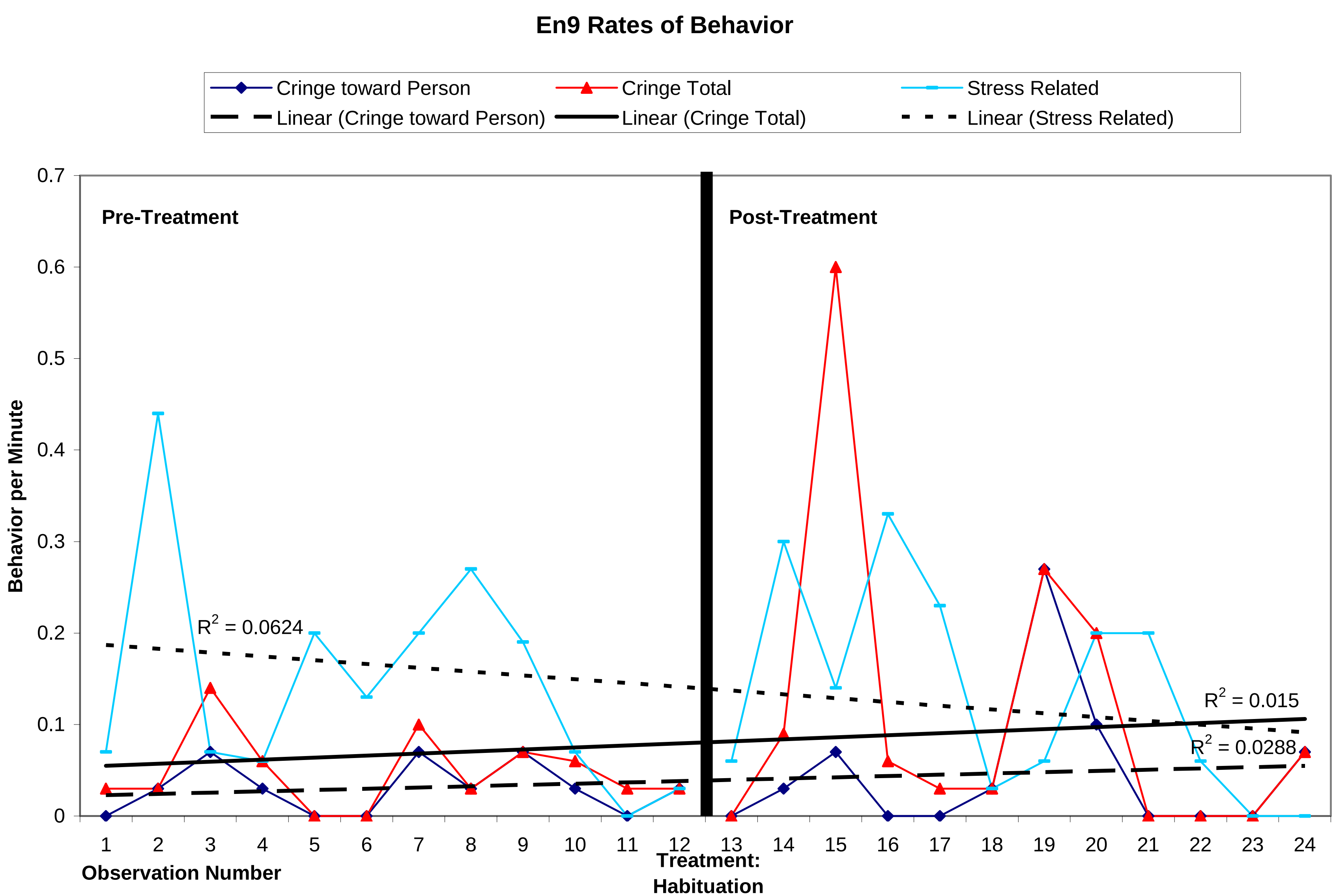

Sg9 Rates of Behavior
Cringe toward Person
Cringe Total
Stress Related
Linear (Cringe toward Person)
Linear (Cringe Total)
Linear (Stress Related)
Pre-Treatment
Post-Treatment
R2 = 0.0884
Cringe (total)
R2 = 0.0124
Cringe (person)
R2 = 0.0696
Treatment:
Husbandry
Behavior per Minute
Observation Number
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
-0.1
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24



Uw8 Rates of Behavior
Cringe toward Person
Cringe Total
Stress Related
Linear (Cringe toward Person)
Linear (Cringe Total)
Linear (Stress Related)
Behavior per Minute
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Pre-Treatment
Post-Treatment
R2 = 0.0941
Cringe (total)
R2 = 0.0373
Cringe (person)
R2 = 0.0002
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
Observation Number
Treatment:
Husbandry

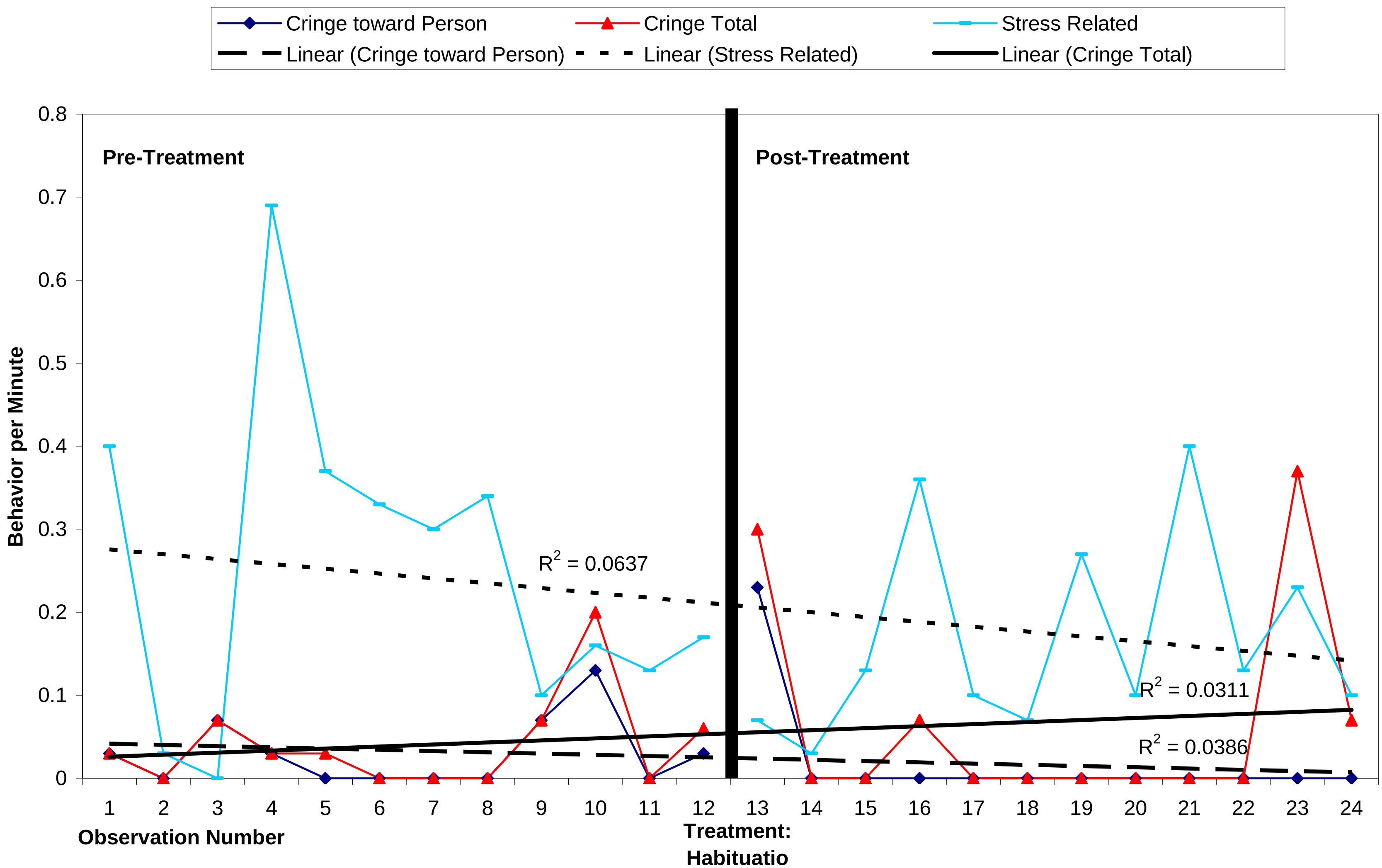
Habituation Group: Rates of Behavior

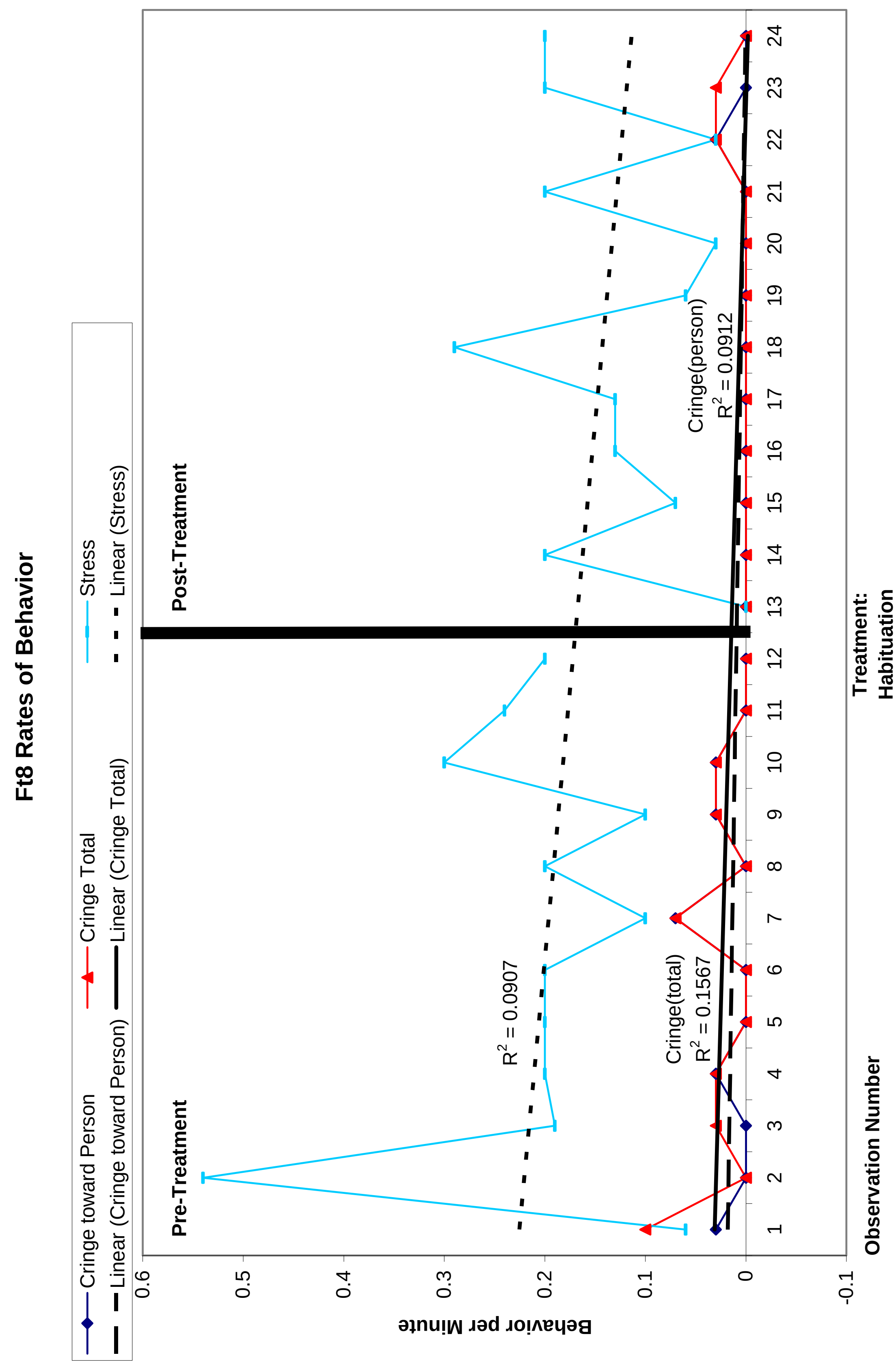


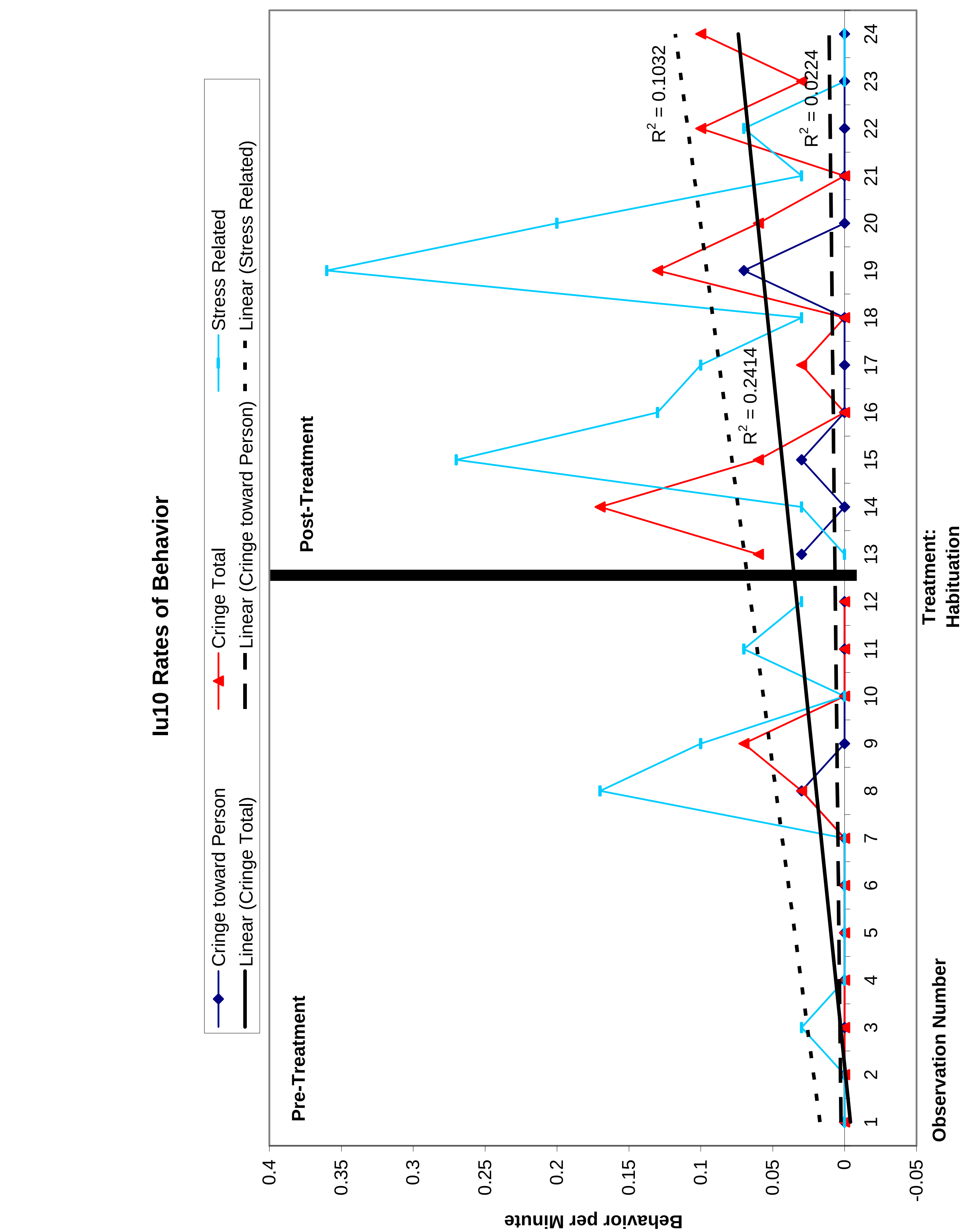


En9 Rates of Behavior
Cringe toward Person
Cringe Total
Stress Related
Linear (Cringe toward Person)
Linear (Cringe Total)
Linear (Stress Related)
Pre-Treatment
Post-Treatment
R2 = 0.0624
R2 = 0.015
R2 = 0.0288
Behavior per Minute
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
Observation Number
Treatment:
Habituation

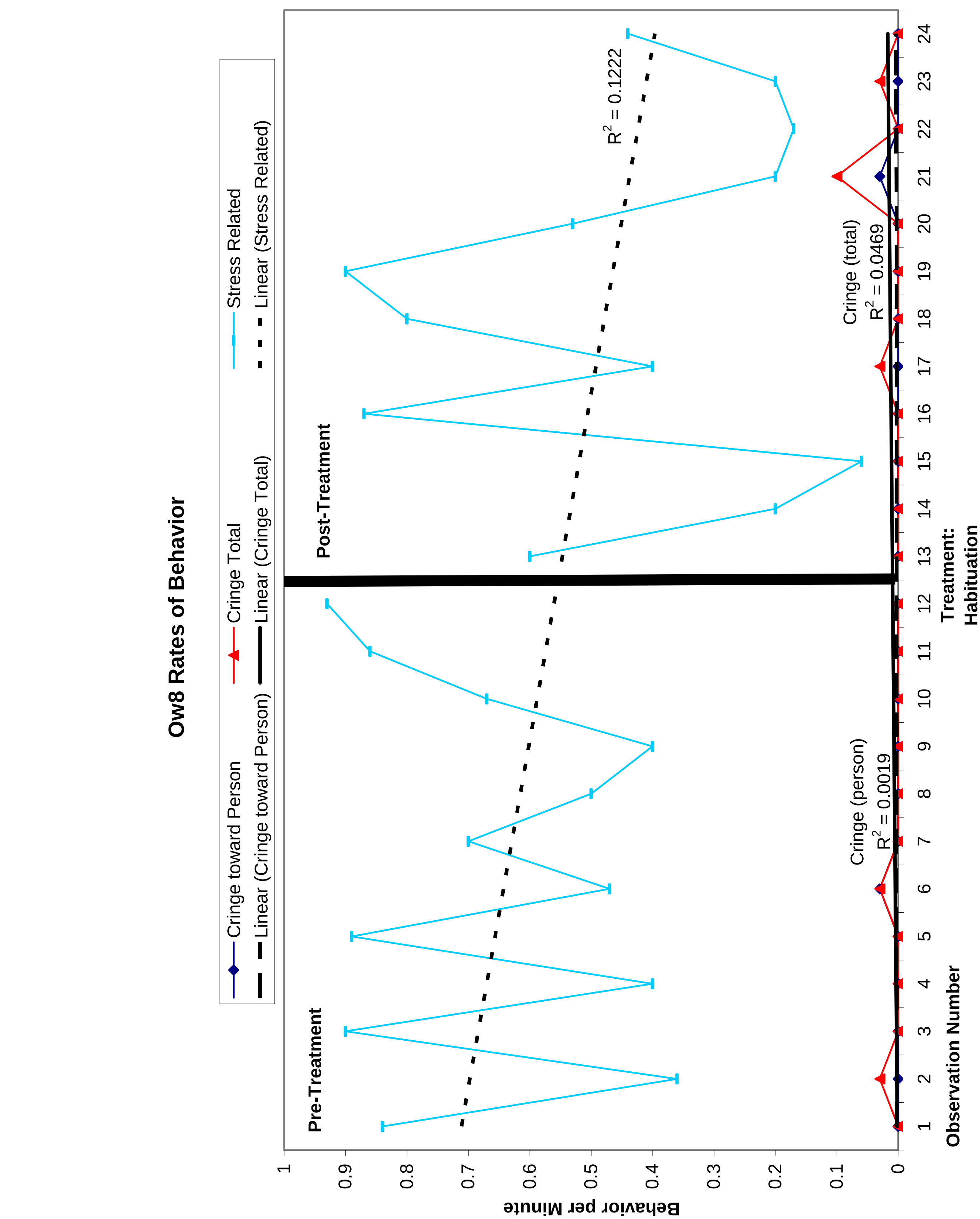
Fp9 Rates of Behavior




Ft8 Rates of Behavior
Cringe toward Person
Cringe Total
Stress
Linear (Cringe toward Person)
Linear (Cringe Total)
Linear (Stress)
Behavior per Minute
0.6
0.5
0.4
0.3
0.2
0.1
0
-0.1
Pre-Treatment
Post-Treatment
$R^2 = 0.0907$
Cringe(total)
$R^2 = 0.1567$
Cringe(person)
$R^2 = 0.0912$
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
Observation Number
Treatment:
Habituation

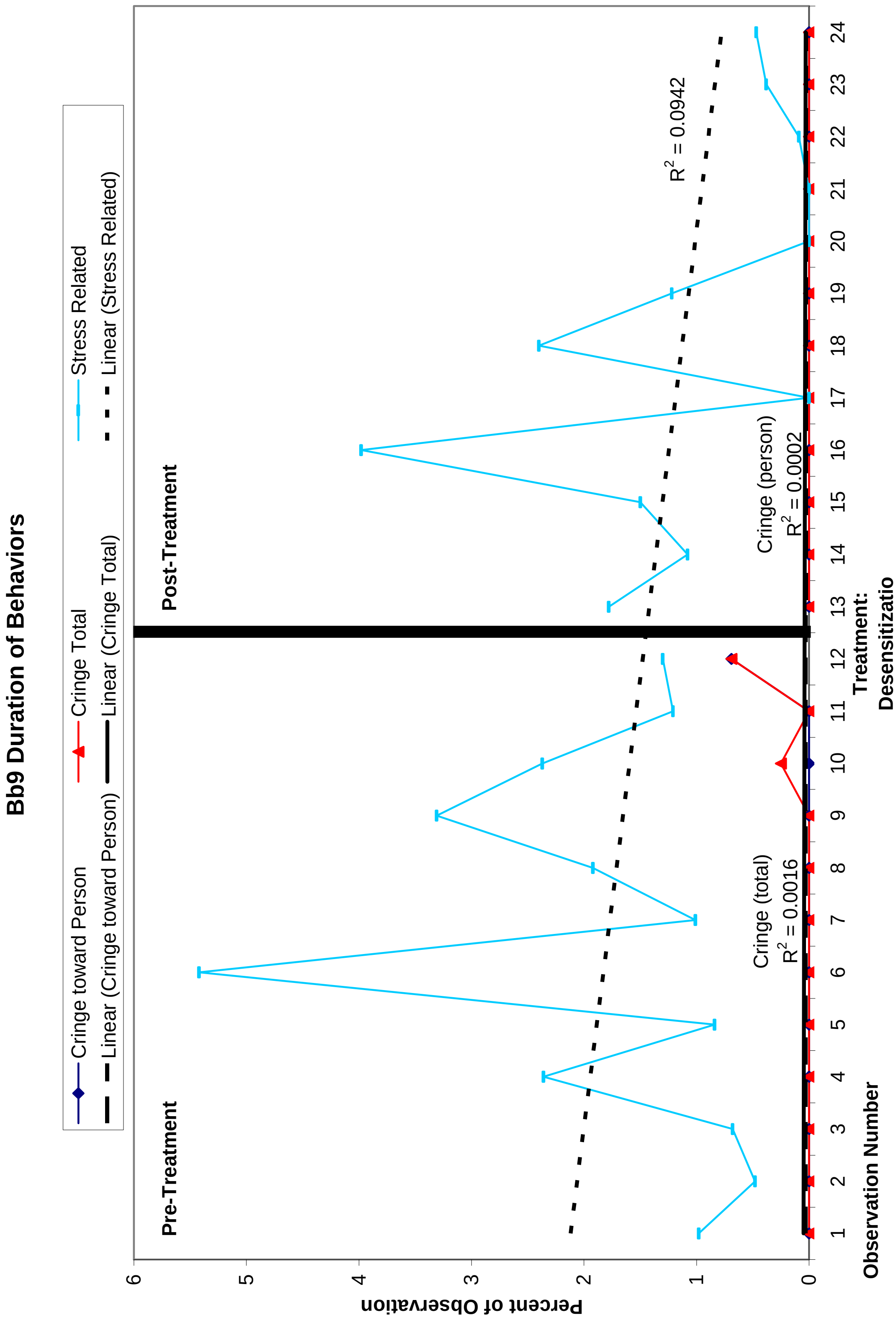


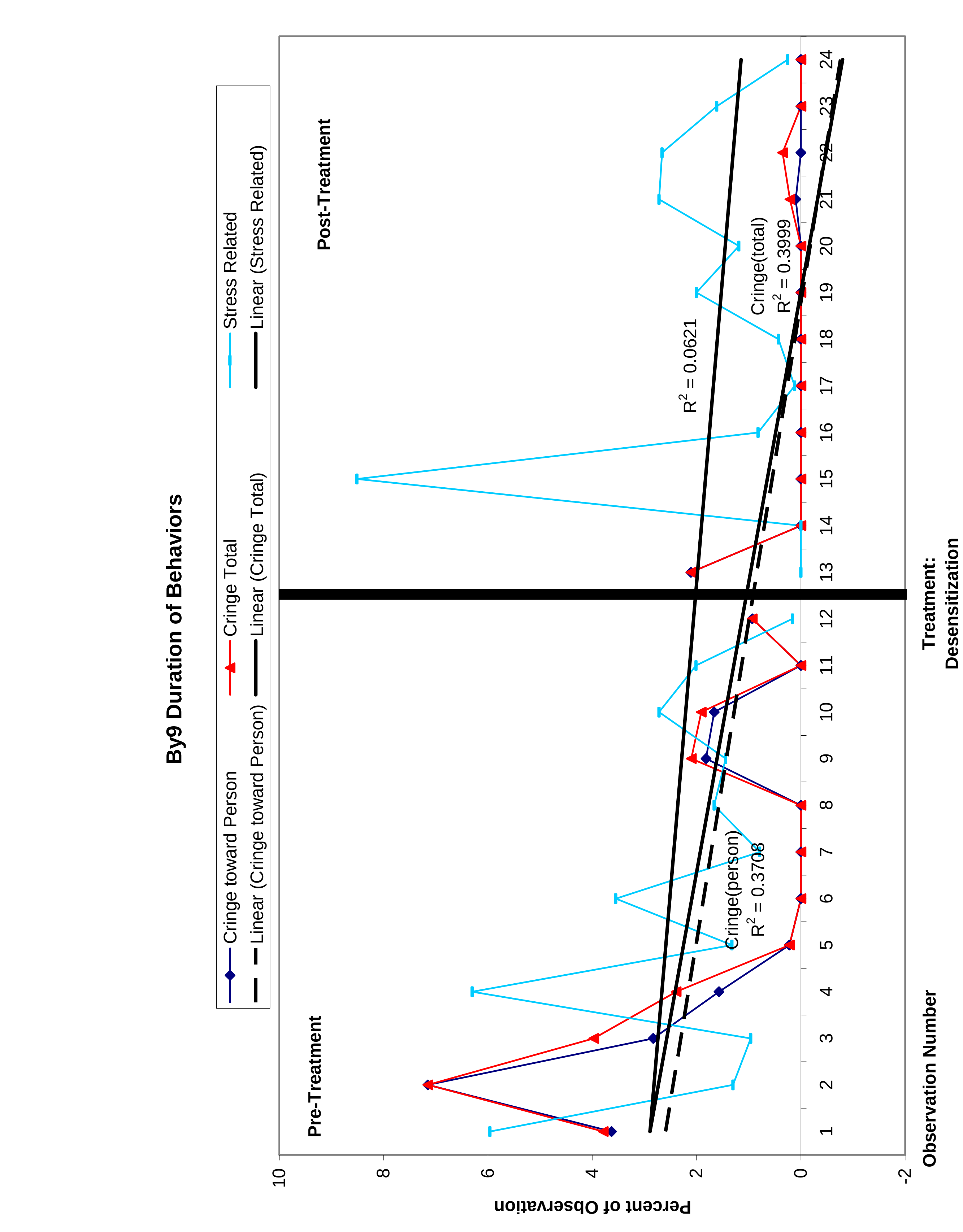
Iu10 Rates of Behavior
Cringe toward Person
Cringe Total
Stress Related
Linear (Cringe Total)
Linear (Cringe toward Person)
Linear (Stress Related)
Pre-Treatment
Post-Treatment
$R^2 = 0.1032$
$R^2 = 0.2414$
$R^2 = 0.0224$
Behavior per Minute
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
-0.05
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
Observation Number
Treatment: Habituation



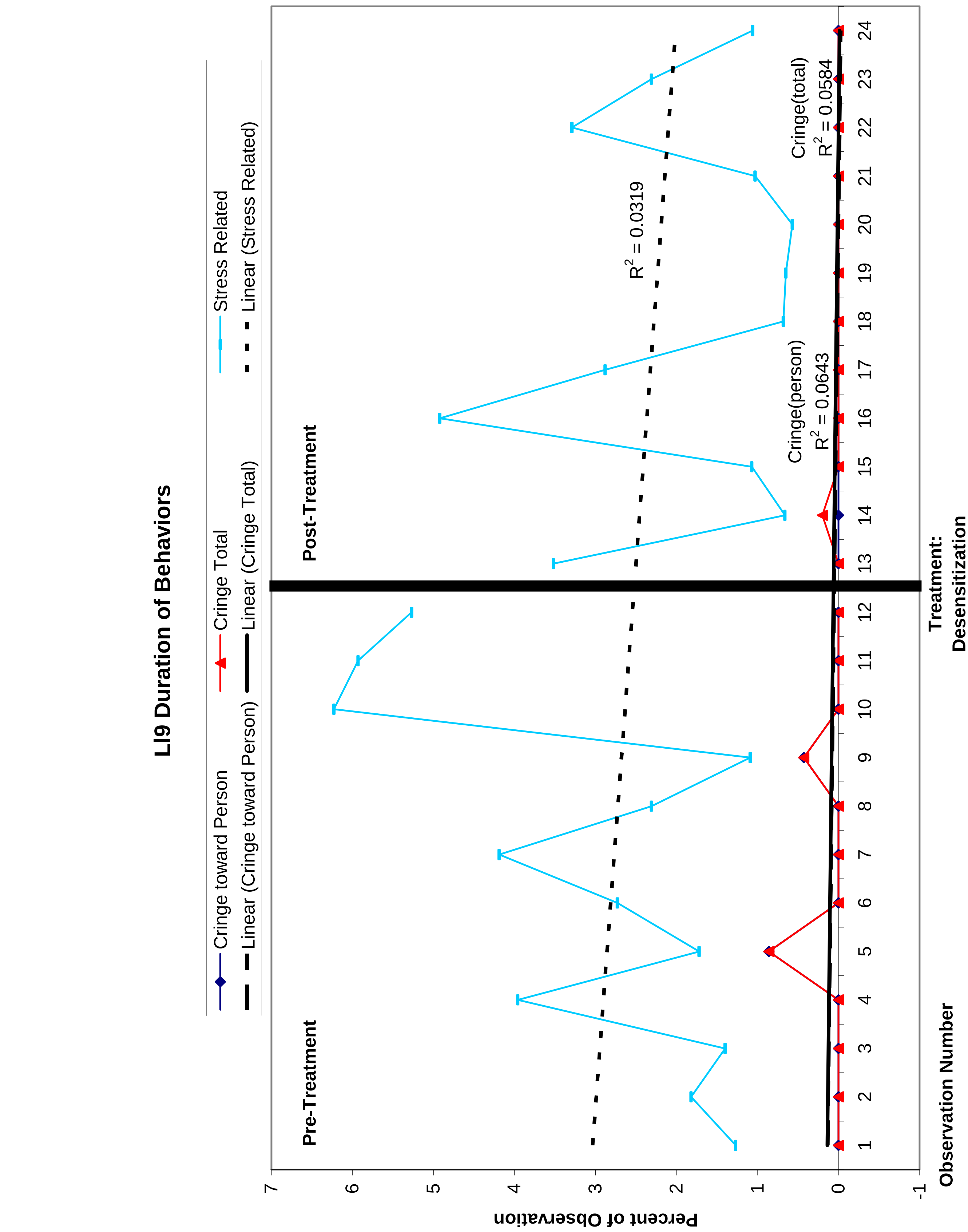
Ow8 Rates of Behavior
Cringe toward Person
Linear (Cringe toward Person)
Cringe Total
Linear (Cringe Total)
Stress Related
Linear (Stress Related)
Behavior per Minute
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Pre-Treatment
Post-Treatment
Cringe (person)
R^2 = 0.0019
Cringe (total)
R^2 = 0.0469
R^2 = 0.1222
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
Observation Number
Treatment:
Habituation

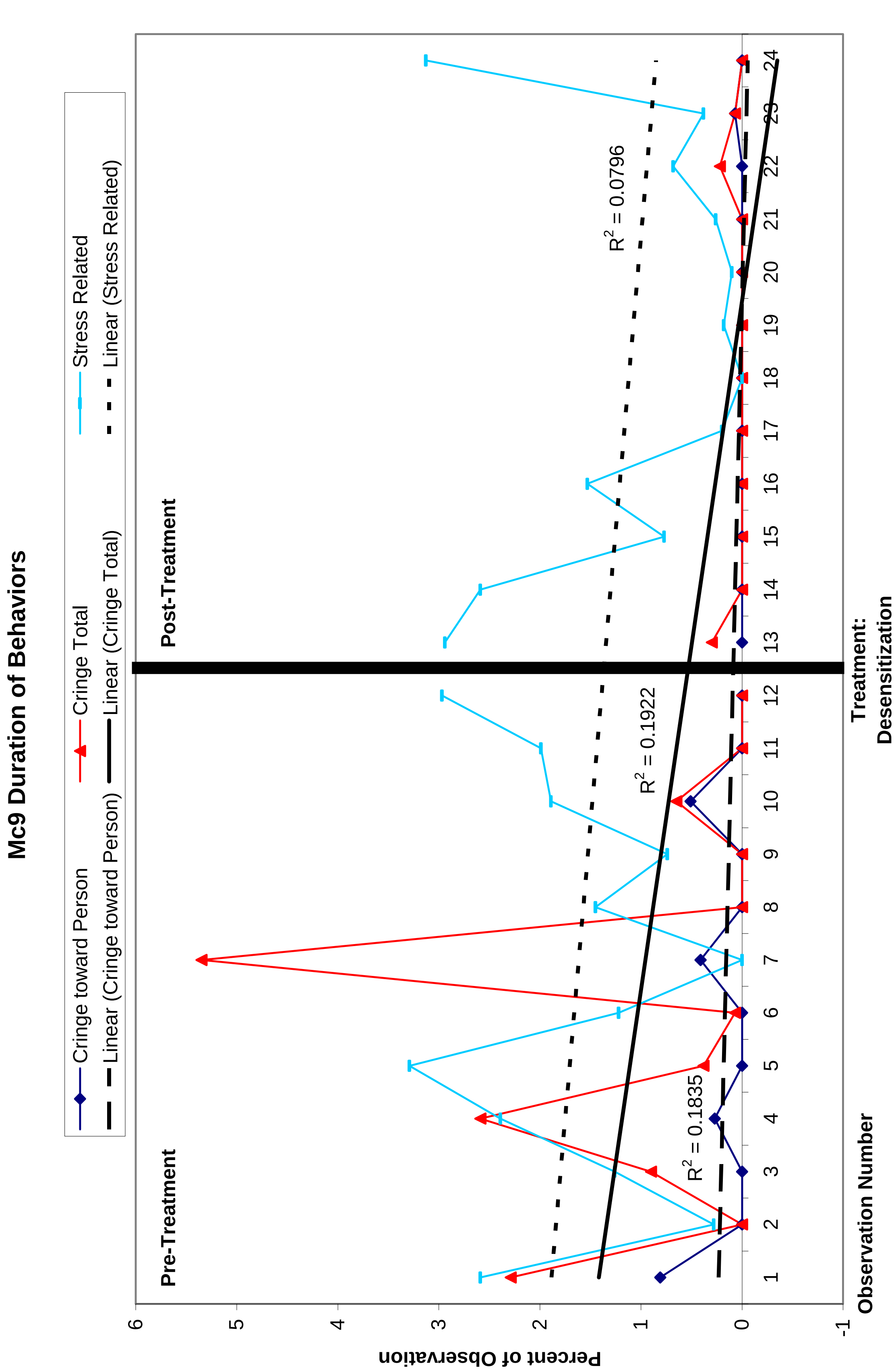
Desensitization Group: Duration of Behaviors

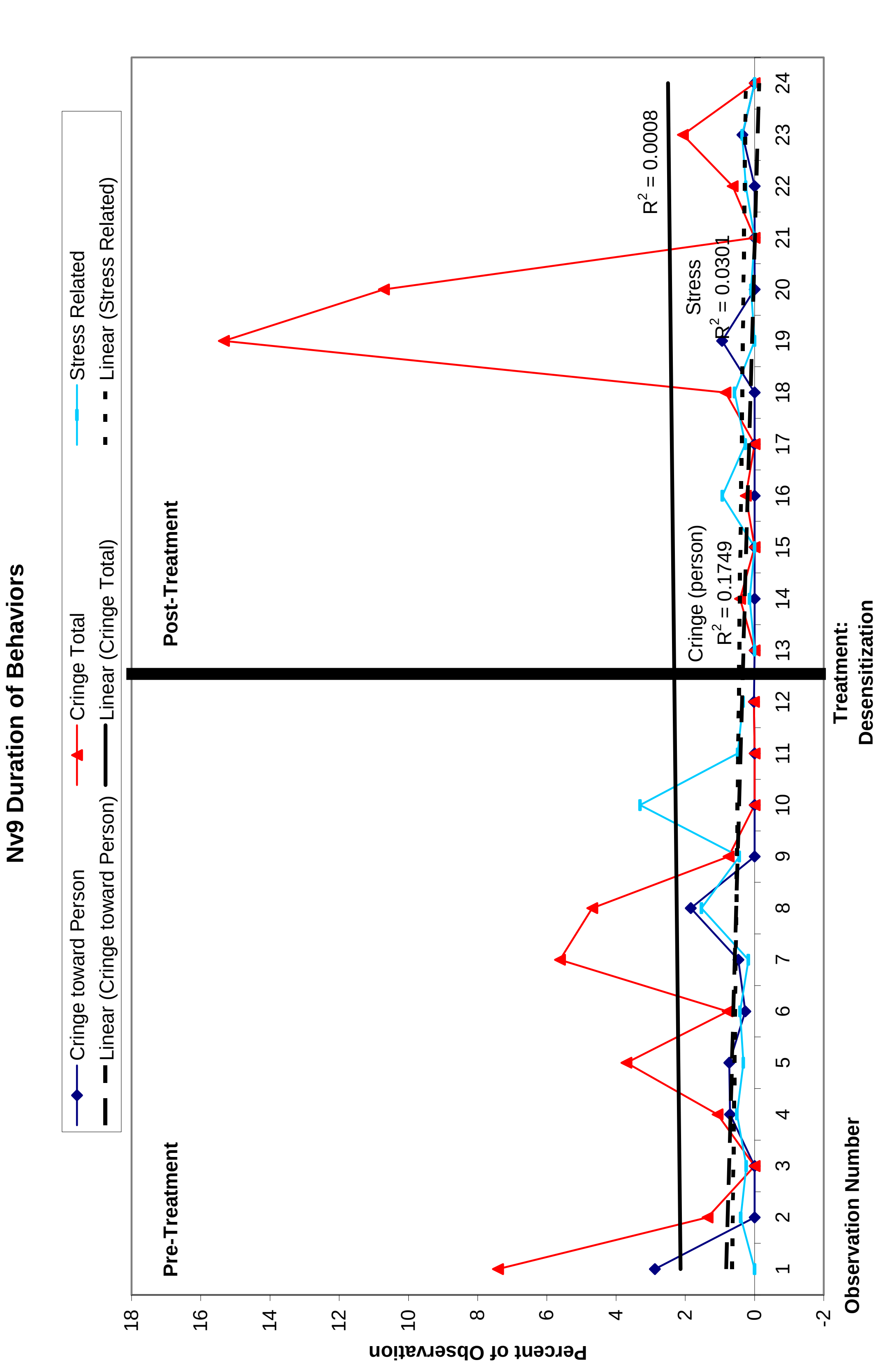




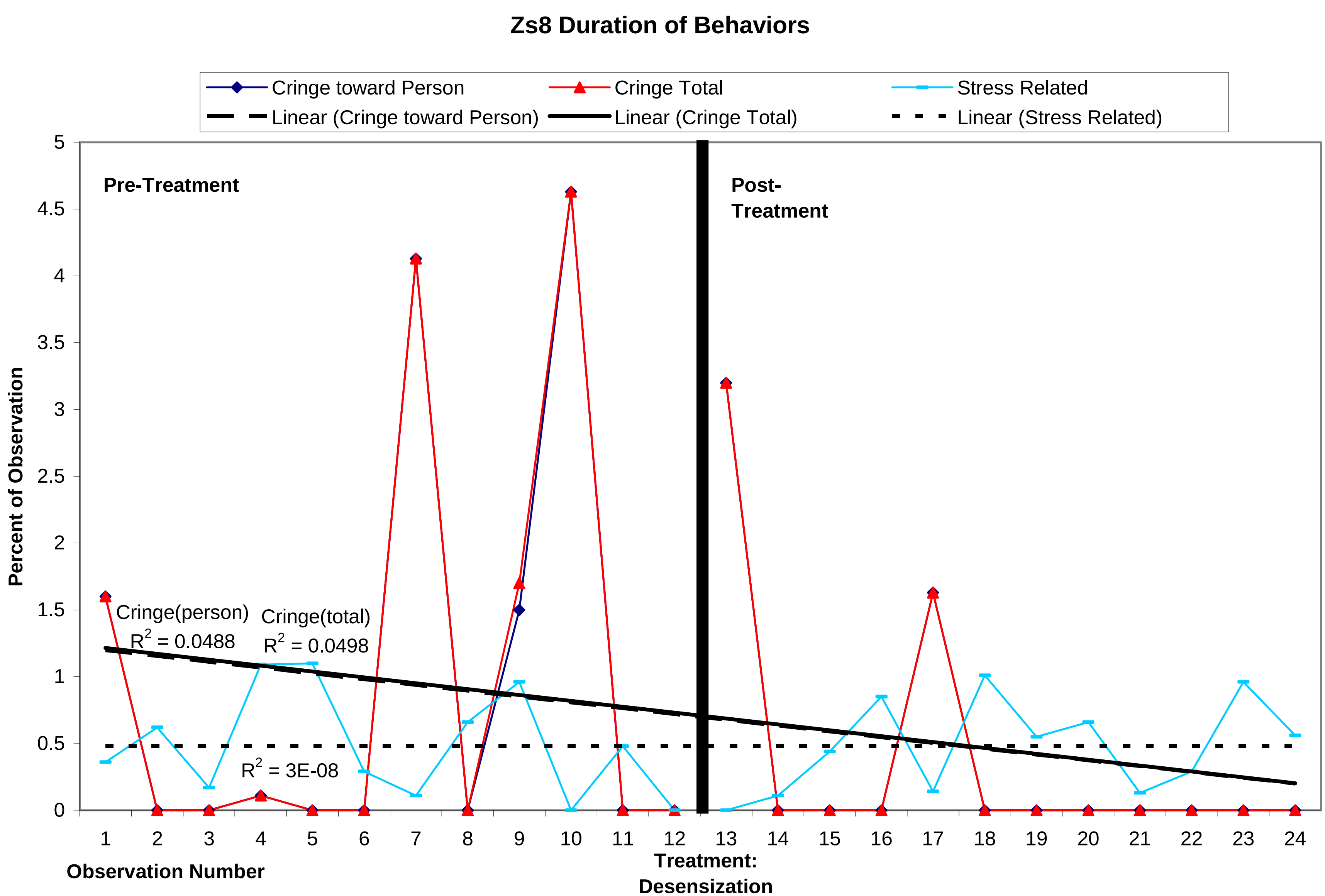
By9 Duration of Behaviors
Cringe toward Person
Cringe Total
Stress Related
Linear (Cringe toward Person)
Linear (Cringe Total)
Linear (Stress Related)
Pre-Treatment
Post-Treatment
Treatment: Desensitization
Percent of Observation
Observation Number
Cringe(person)
$R^2 = 0.3708$
$R^2 = 0.0621$
Cringe(total)
$R^2 = 0.3999$


LI9 Duration of Behaviors
Cringe toward Person
Cringe Total
Stress Related
Linear (Cringe toward Person)
Linear (Cringe Total)
Linear (Stress Related)
Pre-Treatment
Post-Treatment
Treatment: Desensitization
Percent of Observation
Observation Number
$R^2 = 0.0319$
Cringe(person) $R^2 = 0.0643$
Cringe(total) $R^2 = 0.0584$
-1
0
1
2
3
4
5
6
7
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24


Mc9 Duration of Behaviors
Cringe toward Person
Cringe Total
Stress Related
Linear (Cringe toward Person)
Linear (Cringe Total)
Linear (Stress Related)
Pre-Treatment
Post-Treatment
$R^2 = 0.1835$
$R^2 = 0.1922$
$R^2 = 0.0796$
Percent of Observation
Observation Number
Treatment:
Desensitization
6
5
4
3
2
1
0
-1
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24

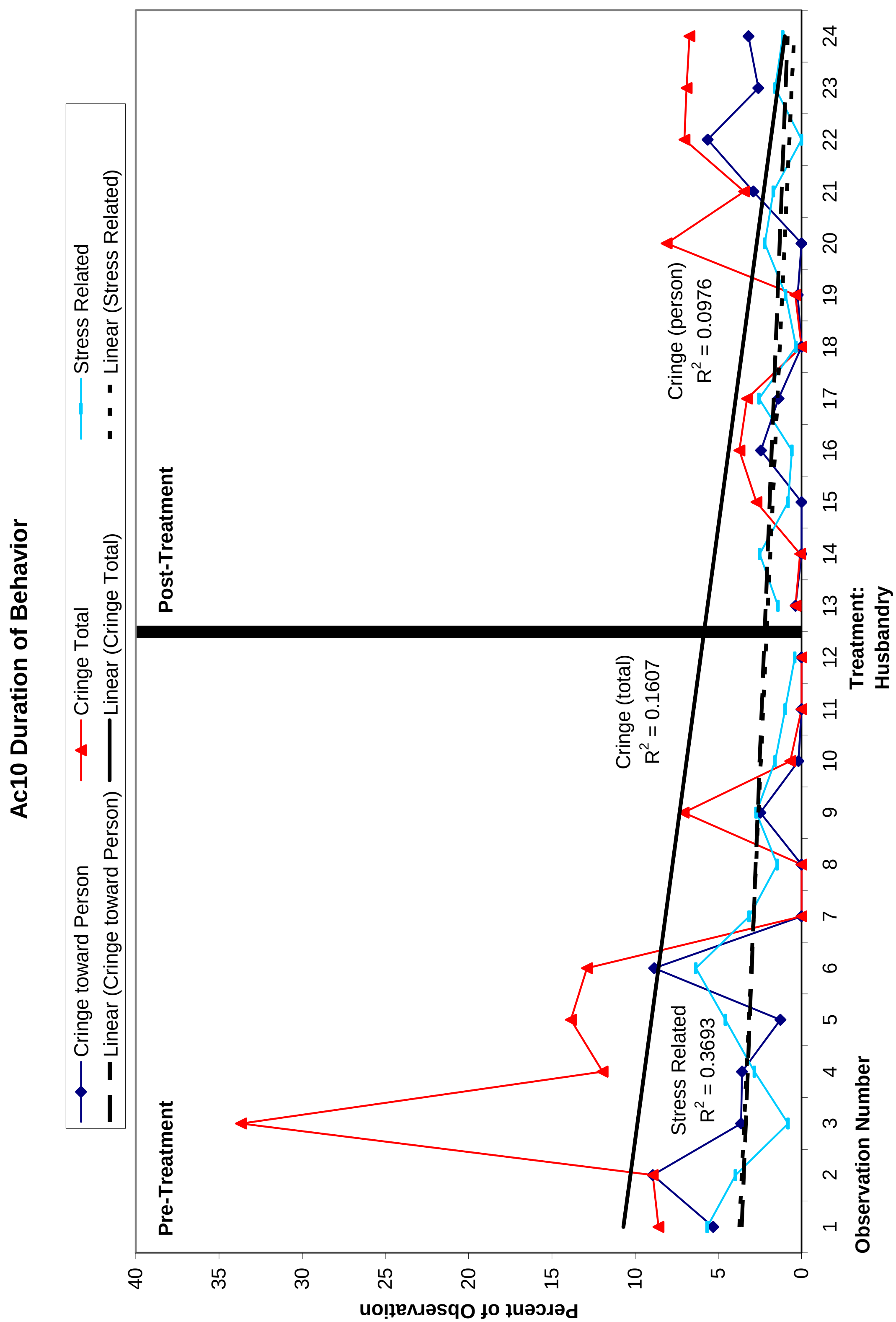


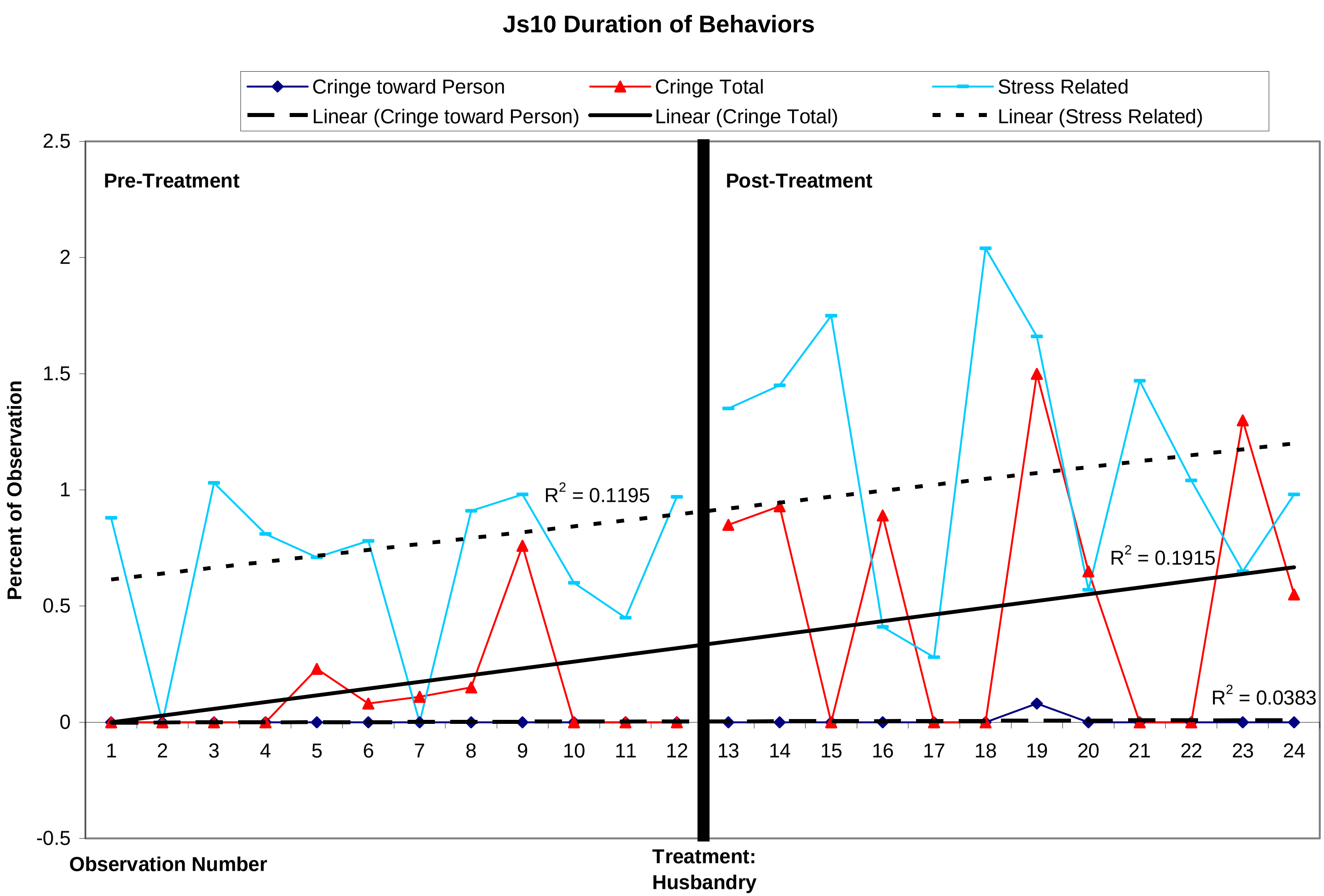
Nv9 Duration of Behaviors
Cringe toward Person
Cringe Total
Stress Related
Linear (Cringe toward Person)
Linear (Cringe Total)
Linear (Stress Related)
Pre-Treatment
Post-Treatment
Cringe (person)
$R^2 = 0.1749$
Stress
$R^2 = 0.0301$
$R^2 = 0.0008$
Treatment:
Desensitization
Percent of Observation
Observation Number



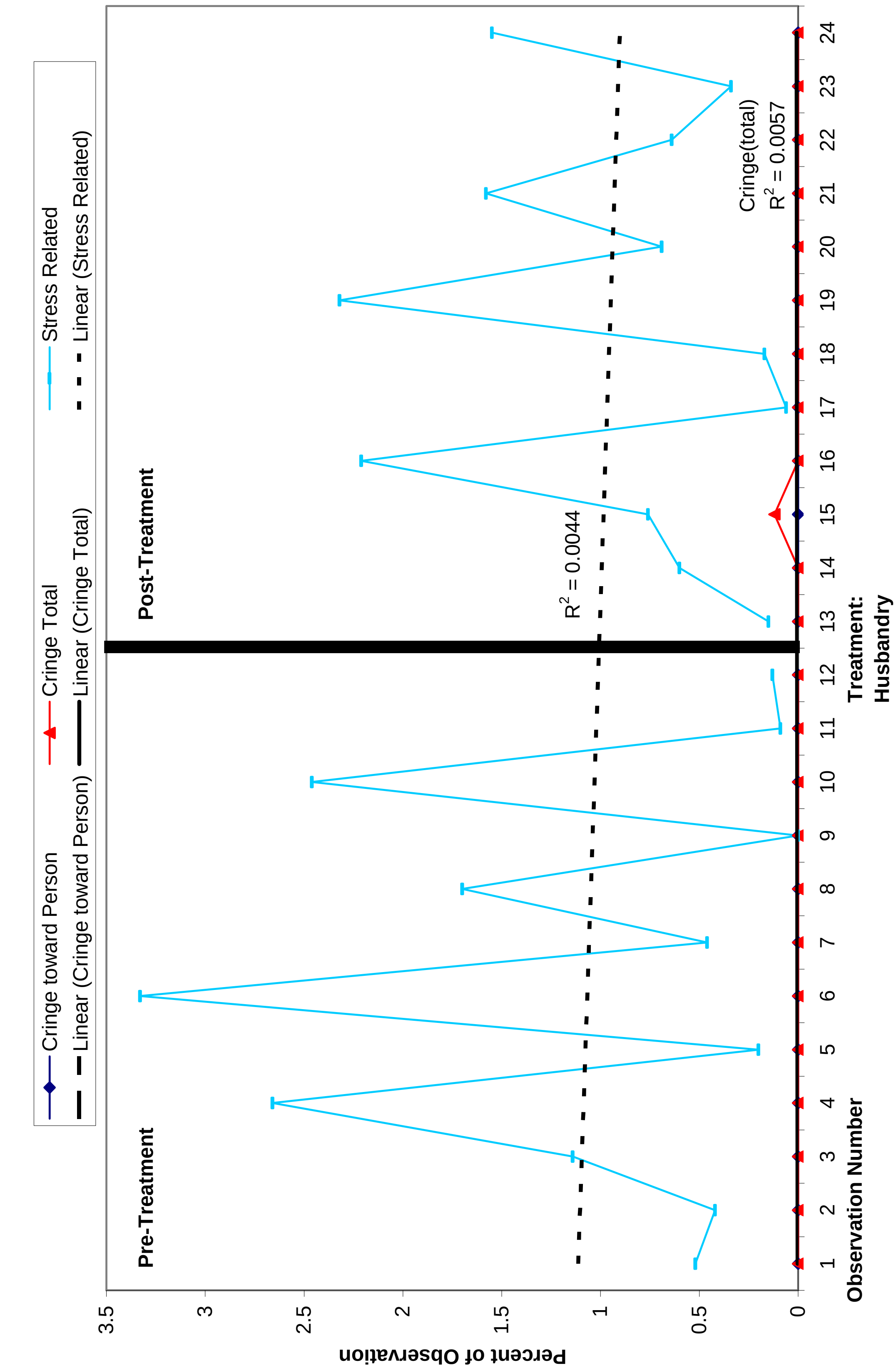
Zs8 Duration of Behaviors
Cringe toward Person
Cringe Total
Stress Related
Linear (Cringe toward Person)
Linear (Cringe Total)
Linear (Stress Related)
Pre-Treatment
Post-
Treatment
Cringe(person)
R2 = 0.0488
Cringe(total)
R2 = 0.0498
R2 = 3E-08
Percent of Observation
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
Observation Number
Treatment:
Desensization

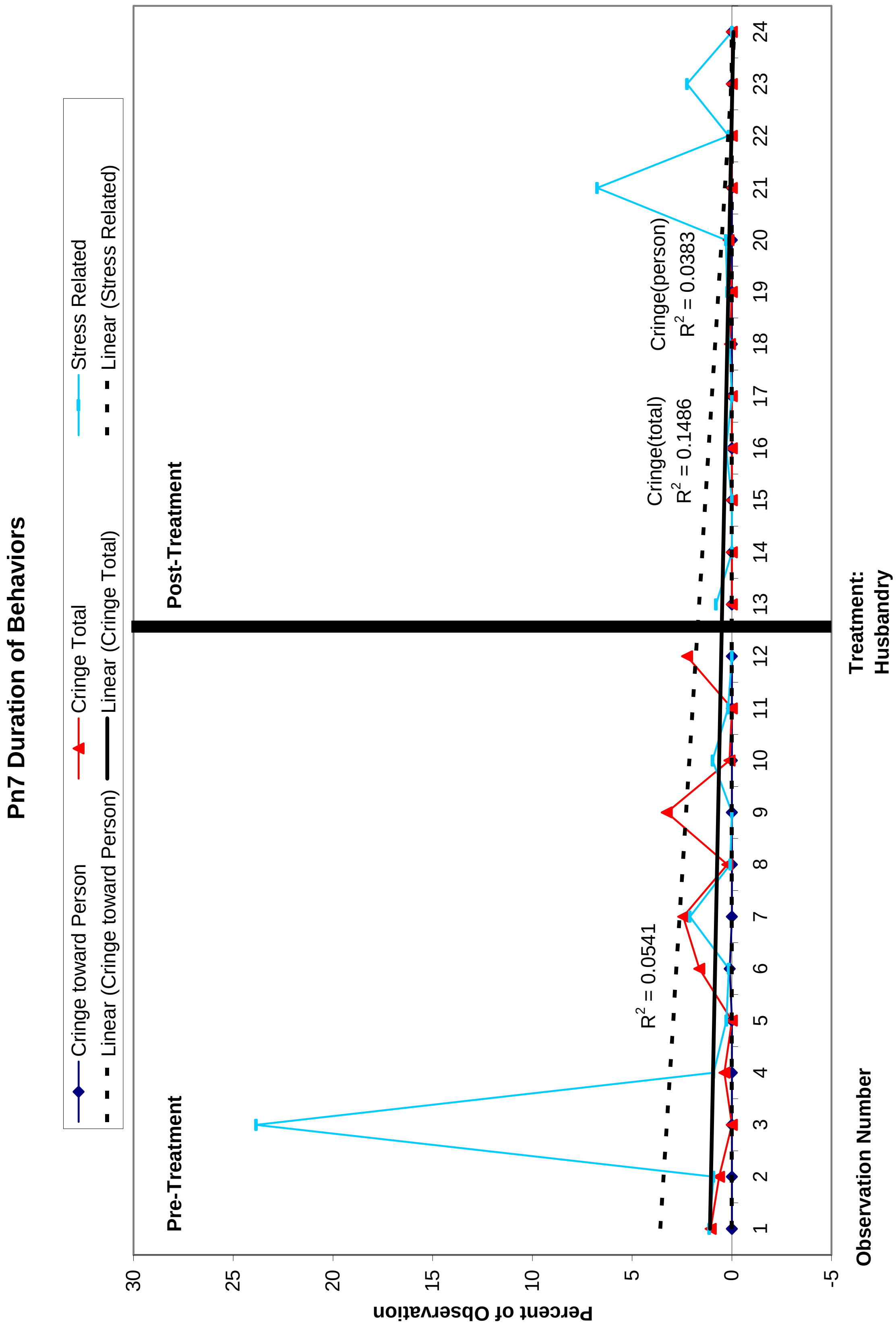
Husbandry Group: Duration of Behaviors



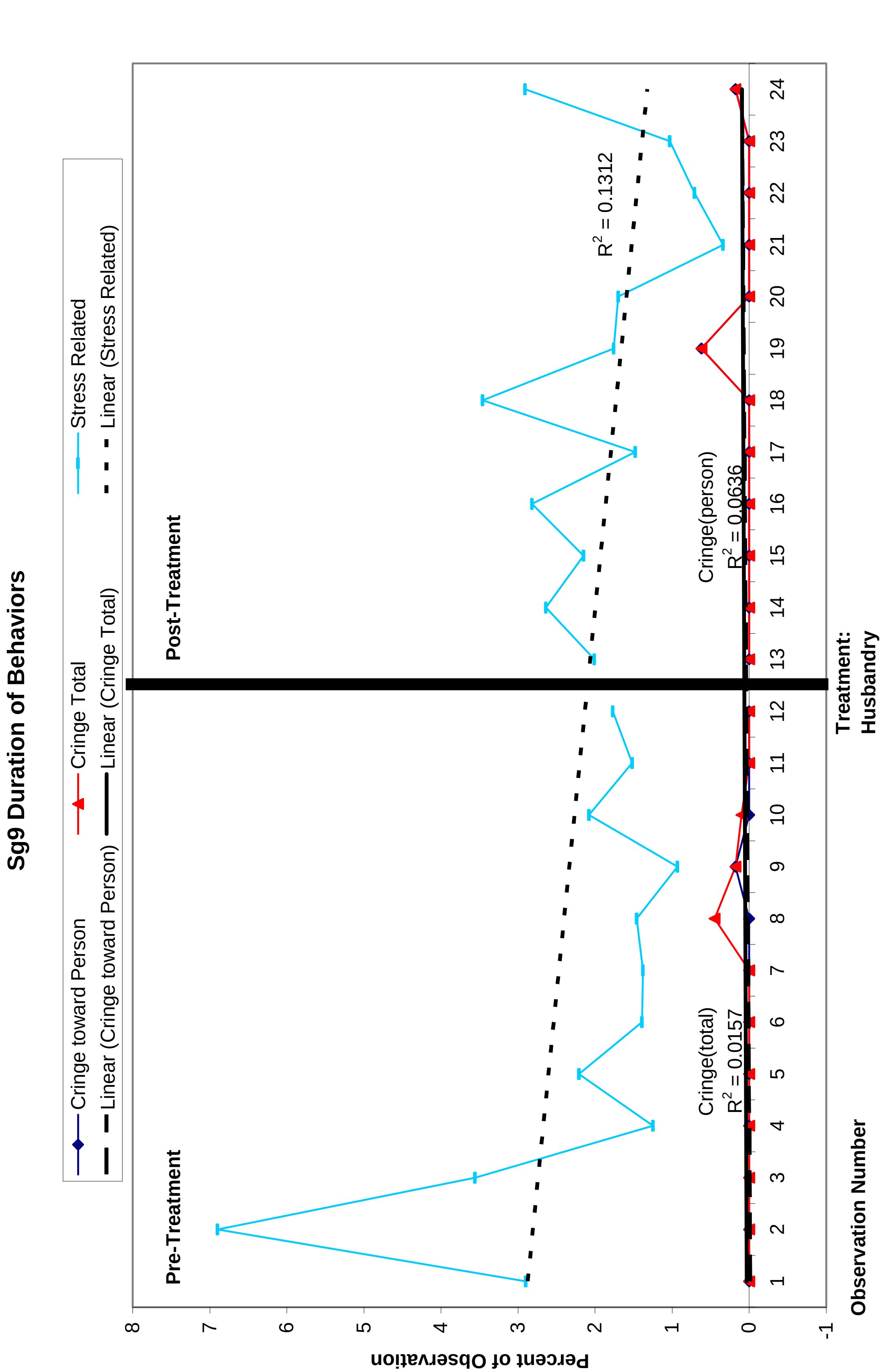

Js10 Duration of Behaviors
Cringe toward Person
Cringe Total
Stress Related
Linear (Cringe toward Person)
Linear (Cringe Total)
Linear (Stress Related)
Pre-Treatment
Post-Treatment
$R^2 = 0.1195$
$R^2 = 0.1915$
$R^2 = 0.0383$
Percent of Observation
Observation Number
Treatment:
Husbandry

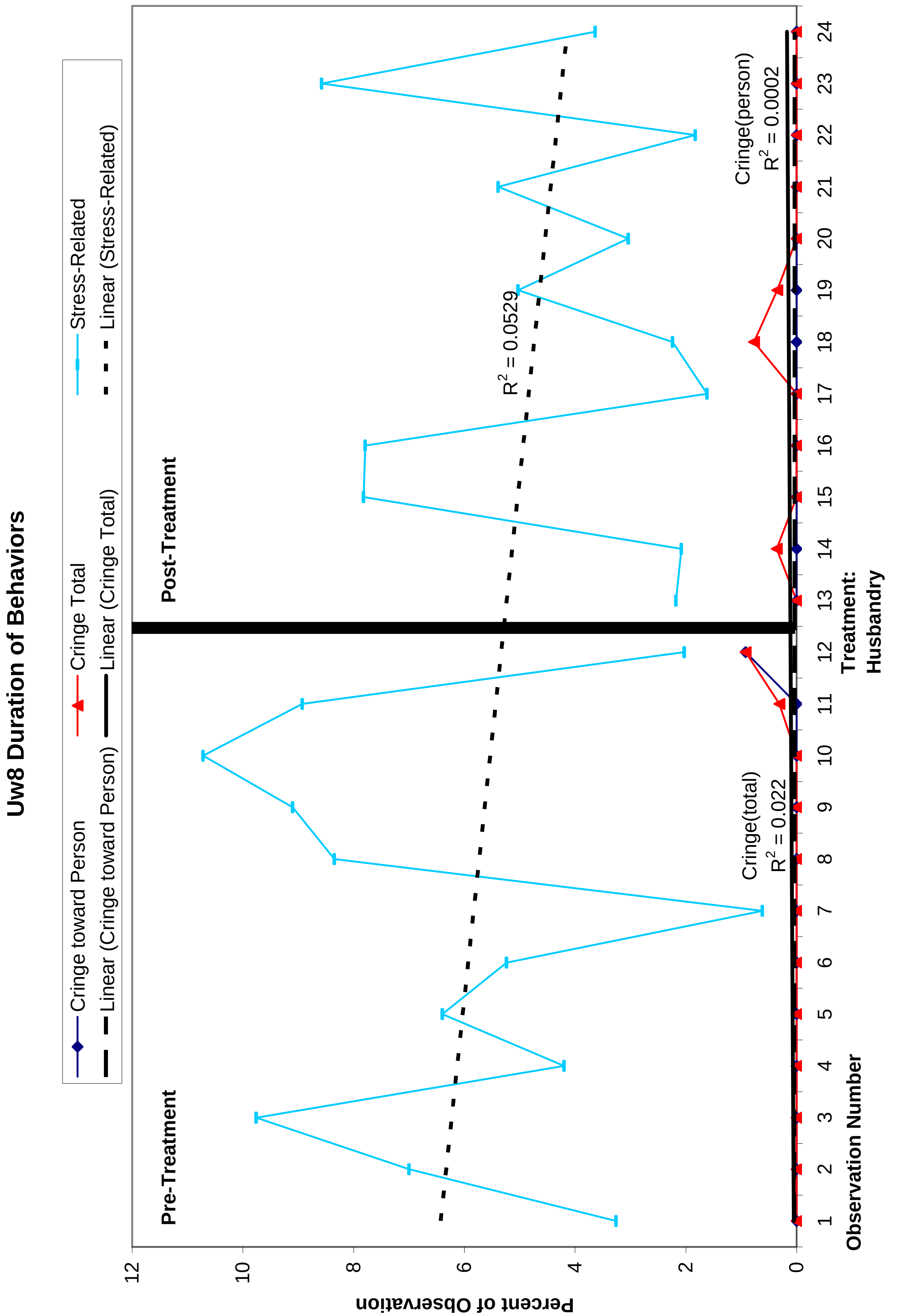
Ne9 Duration of Behaviors



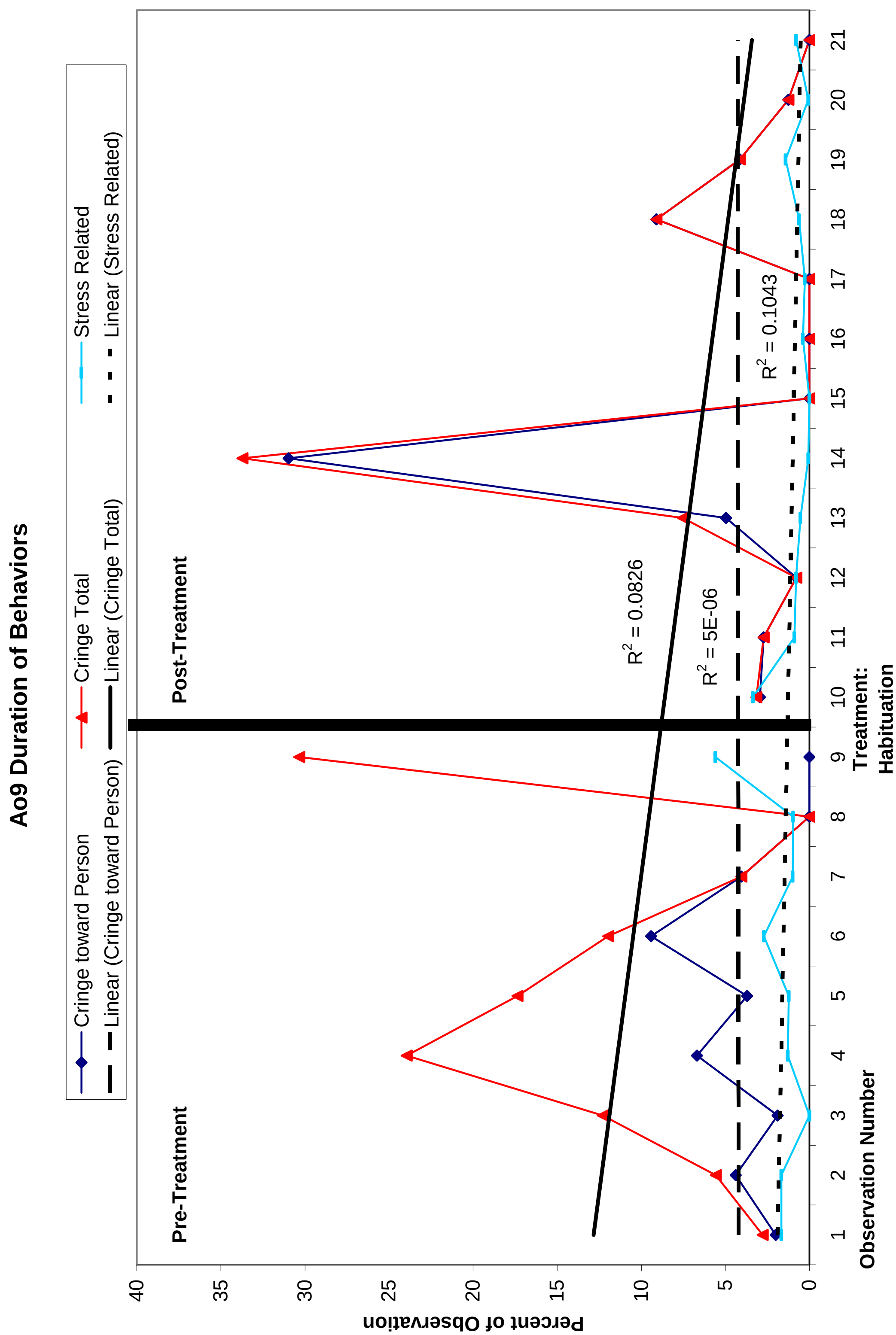


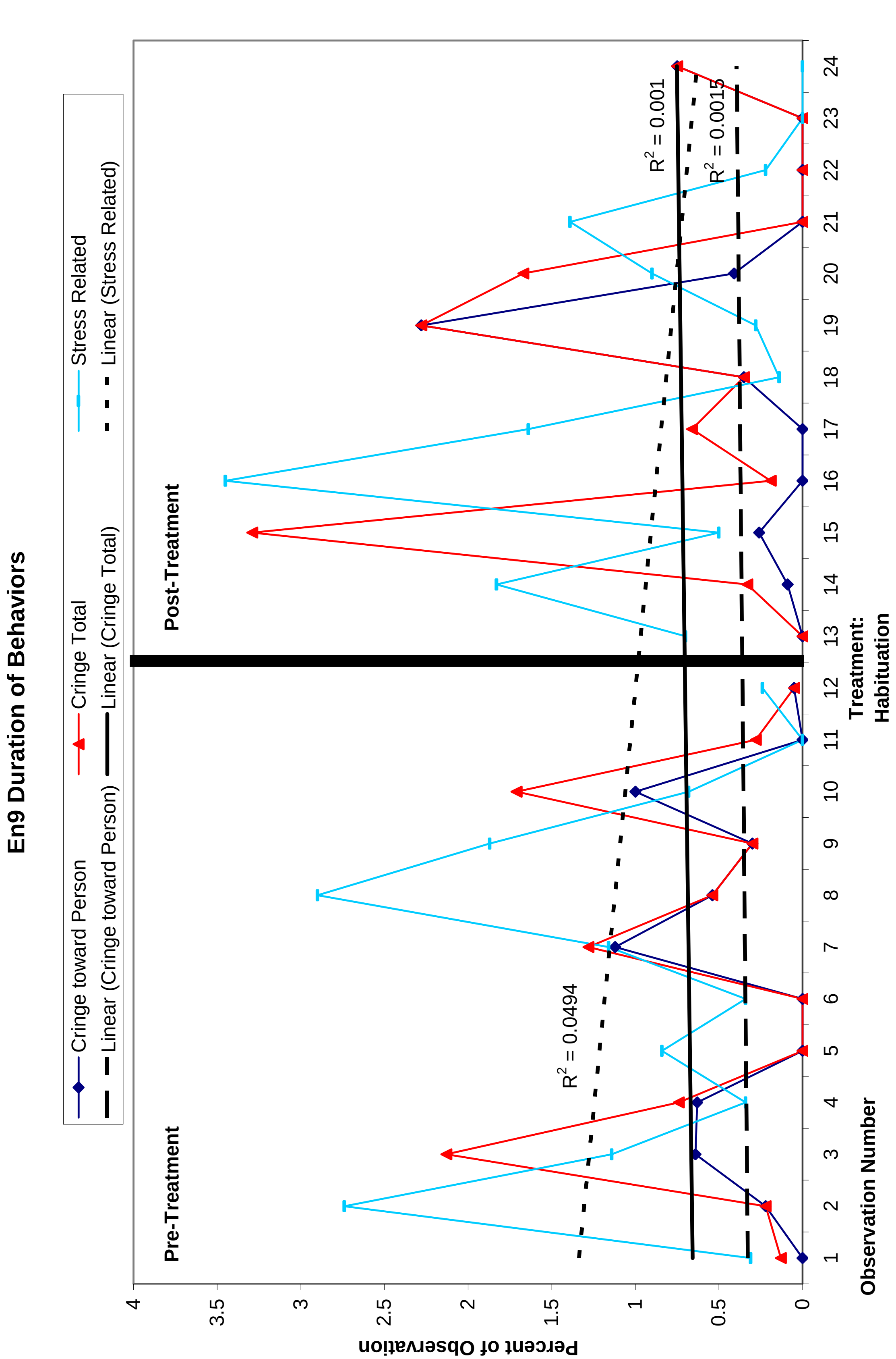
Pn7 Duration of Behaviors
Cringe toward Person
Cringe Total
Stress Related
Linear (Cringe toward Person)
Linear (Cringe Total)
Linear (Stress Related)
Pre-Treatment
Post-Treatment
Percent of Observation
Observation Number
Treatment: Husbandry
R2 = 0.0541
Cringe(total)
R2 = 0.1486
Cringe(person)
R2 = 0.0383
30
25
20
15
10
5
0
-5
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24


Sg9 Duration of Behaviors
Cringe toward Person
Cringe Total
Stress Related
Linear (Cringe toward Person)
Linear (Cringe Total)
Linear (Stress Related)
Pre-Treatment
Post-Treatment
Cringe(total)
R2 = 0.0157
Cringe(person)
R2 = 0.0636
R2 = 0.1312
Treatment:
Husbandry
Percent of Observation
Observation Number
8
7
6
5
4
3
2
1
0
-1
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24

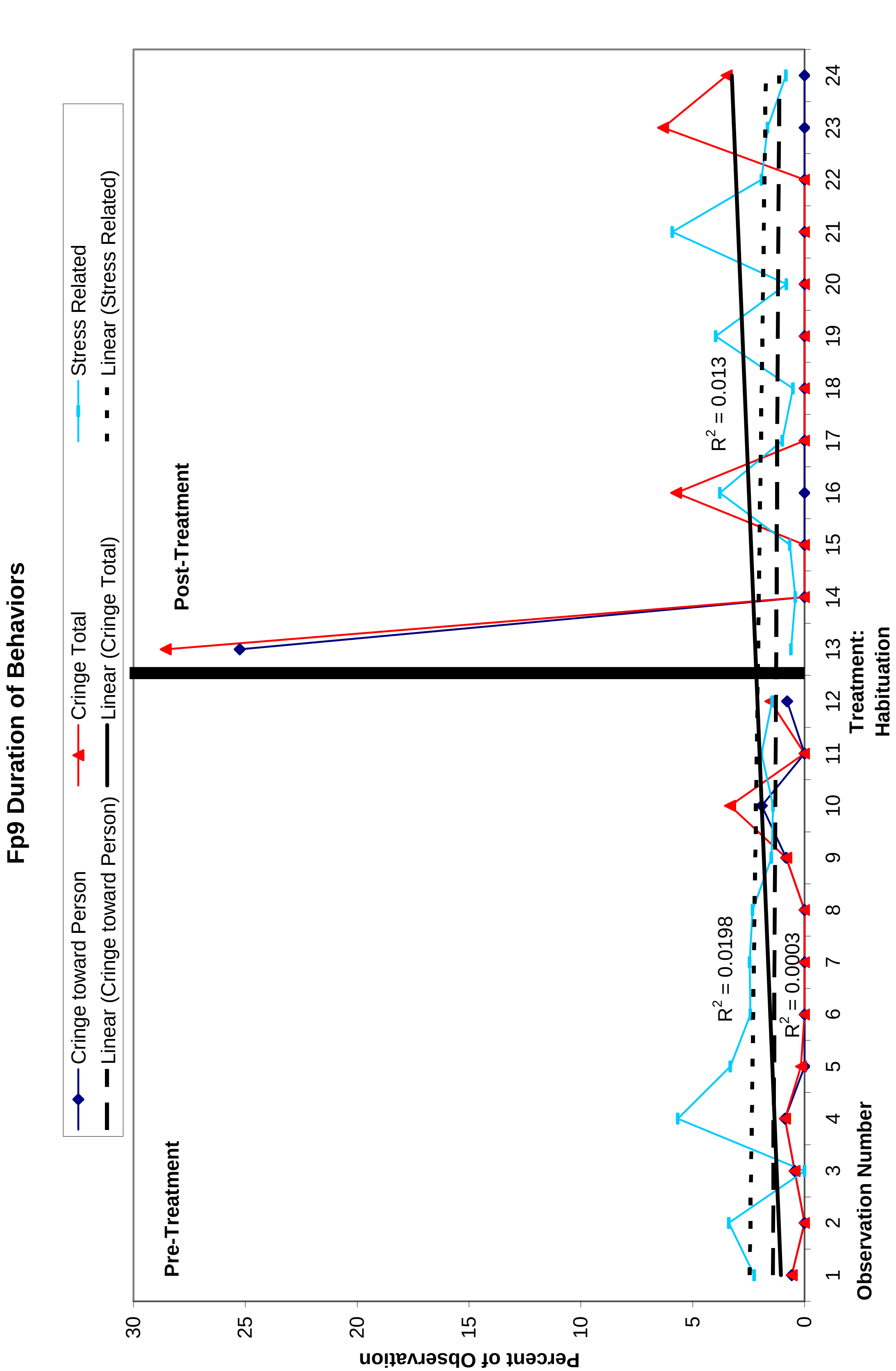

Uw8 Duration of Behaviors
Cringe toward Person
Cringe Total
Stress-Related
Linear (Cringe toward Person)
Linear (Cringe Total)
Linear (Stress-Related)
Pre-Treatment
Post-Treatment
Cringe(total)
R2 = 0.022
R2 = 0.0529
Cringe(person)
R2 = 0.0002
Percent of Observation
Observation Number
Treatment:
Husbandry
0
2
4
6
8
10
12
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24

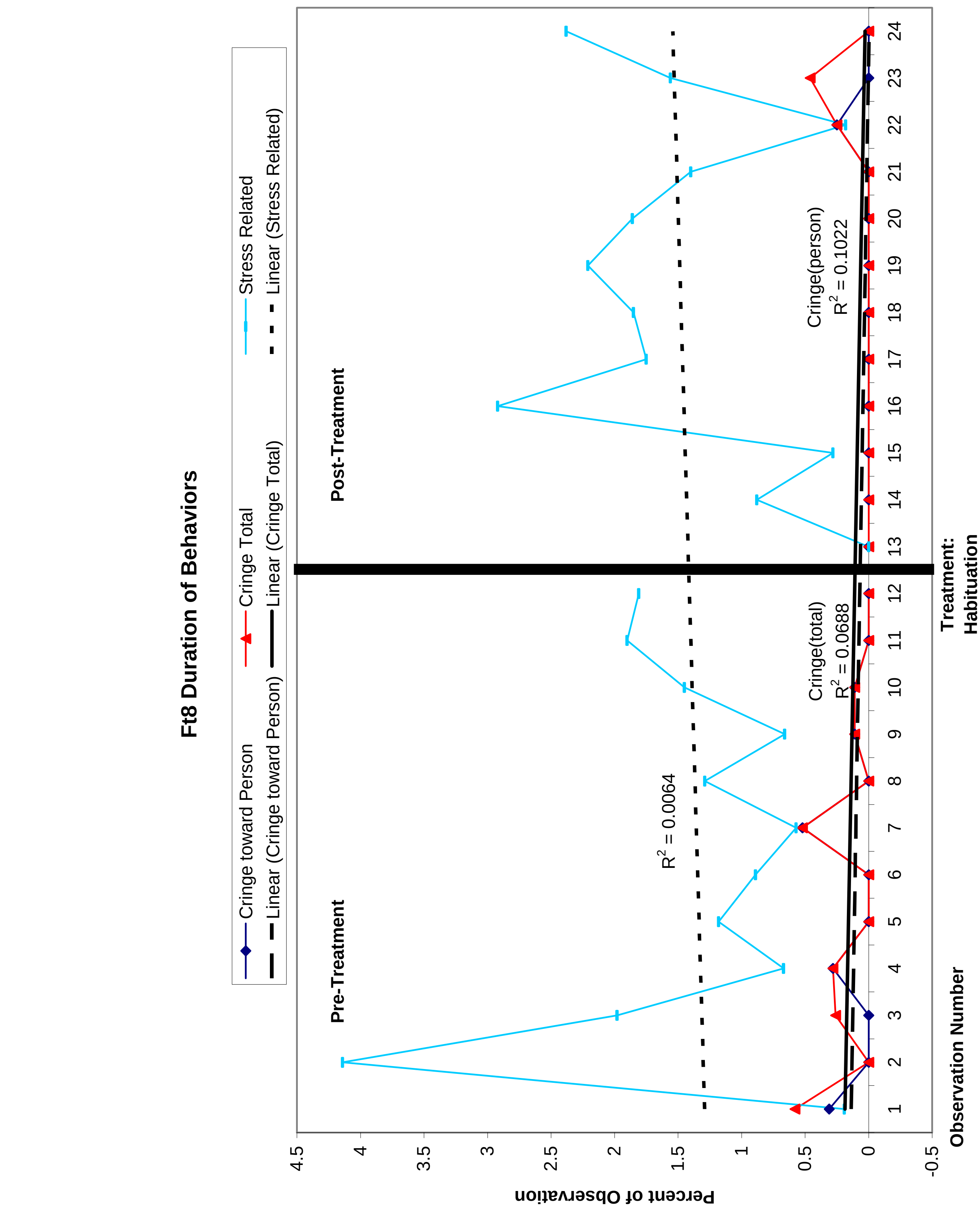
Habituation Group: Duration of Behaviors





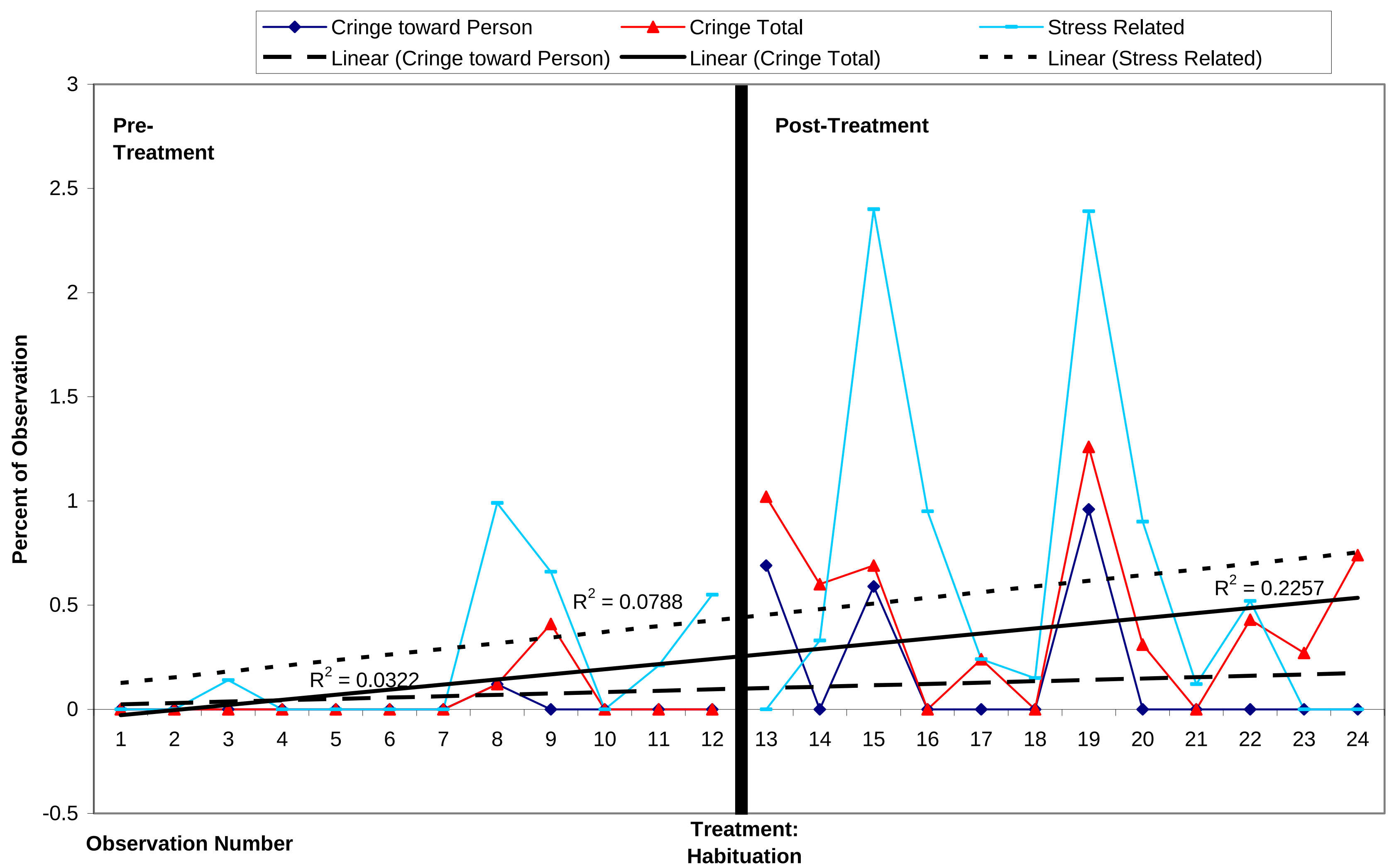
En9 Duration of Behaviors
Cringe toward Person
Cringe Total
Stress Related
Linear (Cringe toward Person)
Linear (Cringe Total)
Linear (Stress Related)
Pre-Treatment
Post-Treatment
$R^2 = 0.0494$
$R^2 = 0.001$
$R^2 = 0.0015$
Percent of Observation
0
0.5
1
1.5
2
2.5
3
3.5
4
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
Observation Number
Treatment: Habituation

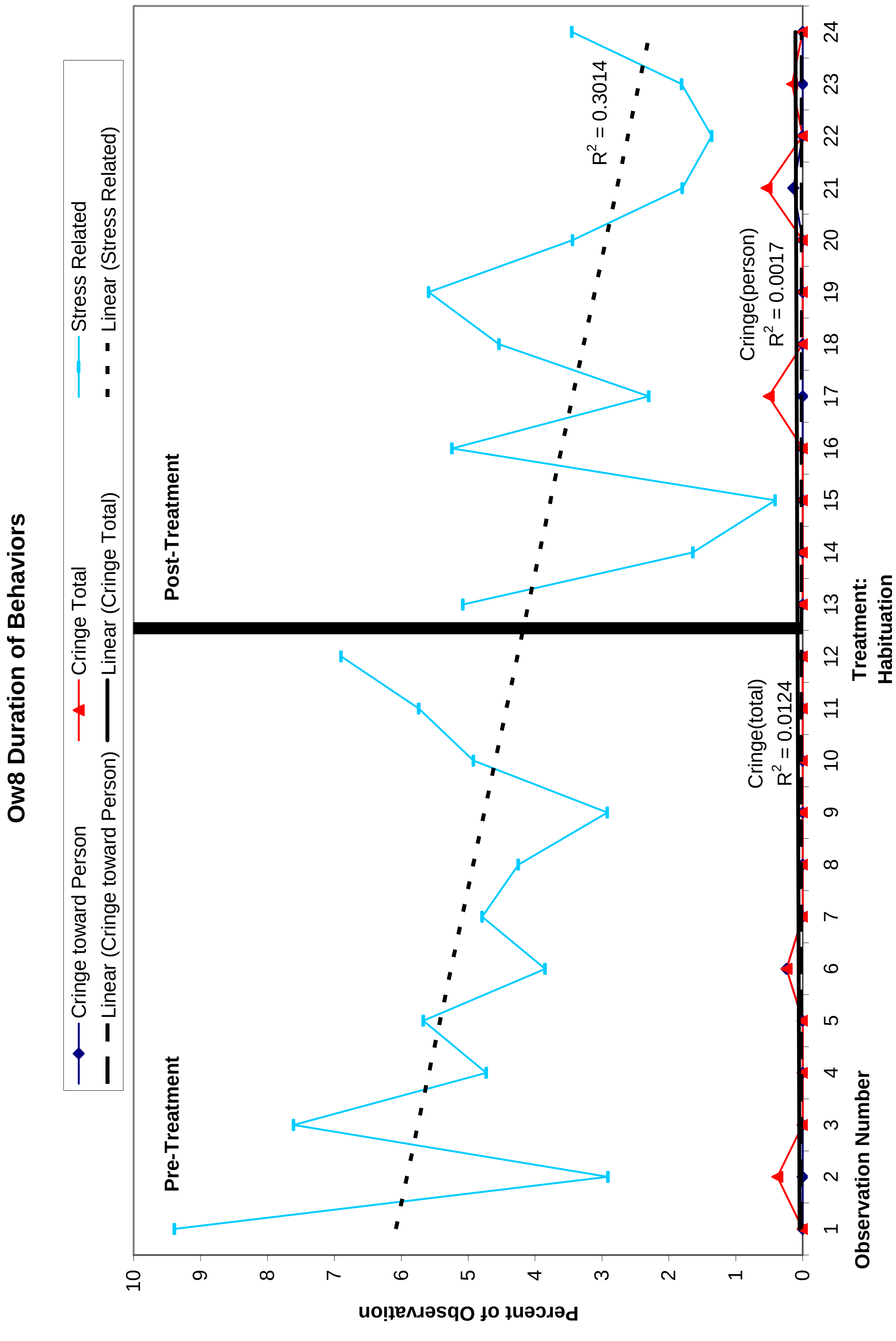

Fp9 Duration of Behaviors
Cringe toward Person
Cringe Total
Stress Related
Linear (Cringe toward Person)
Linear (Cringe Total)
Linear (Stress Related)
Pre-Treatment
Post-Treatment
$R^2 = 0.0198$
$R^2 = 0.0003$
$R^2 = 0.013$
Percent of Observation
Observation Number
Treatment: Habituation



Ft8 Duration of Behaviors
Cringe toward Person
Cringe Total
Stress Related
Linear (Cringe toward Person)
Linear (Cringe Total)
Linear (Stress Related)
Pre-Treatment
Post-Treatment
$R^2 = 0.0064$
Cringe(total)
$R^2 = 0.0688$
Cringe(person)
$R^2 = 0.1022$
Treatment:
Habituation
Observation Number
Percent of Observation

Iu10 Duration of Behaviors




Ow8 Duration of Behaviors
Cringe toward Person
Cringe Total
Stress Related
Linear (Cringe toward Person)
Linear (Cringe Total)
Linear (Stress Related)
Pre-Treatment
Post-Treatment
Percent of Observation
0
1
2
3
4
5
6
7
8
9
10
Observation Number
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
Treatment:
Habituation
Cringe(total)
$R^2 = 0.0124$
Cringe(person)
$R^2 = 0.0017$
$R^2 = 0.3014$

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